WEST Search History

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DATE: Monday, December 09, 2002

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| L13 | L12 and 11 and 12 and 13 and 14 and 16 and 17 | 0 | L13 |
| L12 | phenolic | 60507 | L12 |
| L11 | antioxidant | 67482 | L11 |
| L10 | flax seeds | 176 | L10 |
| L9 | rape seeds | 1374 | L9 |
| L8 | safflower seeds | 436 | L8 |
| L7 | peanuts | 22507 | L7 |
| L6 | cottonseeds | 12801 | L6 |
| L5 | mustard seeds | 263 | L5 |
| L4 | hops | 7320 | L4 |
| L3 | soy beans | 4748 | L3 |
| L2 | sunflower seeds | 2159 | L2 |
| L1 | buckwheat | 1472 | L1 |
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| L7 | L6 and 14 and 13 and 12 and 11 | - 0 | - L7 |
| L6 | vegetable protein | 2547 | L6 |
| L5 | vegetable protein bound phenolics | 0 | L5 |
| L4 | quercetin | 746 | L4 |
| L3 | trolox | 154 | L3 |
| L2 | antioxidants | 67482 | L2 |
| L1 | phenolics | 60507 | L1 |
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Quantitation of Phytoestrogens in Legumes by HPLC†,‡

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A fast, sensitive, and precise method is presented for the efficient extraction and quantitation of coumestrol, daidzein, genistein, formononetin, and biochanin A from foods by diode array reversed-phase HPLC analysis using flavone as internal standard. Acid hydrolysis during extraction of foods was chosen to convert the various phytoestrogen conjugates into their respective aglycons, facilitating HPLC analysis and allowing quantitation of total phytoestrogens as aglycons including originally present glycosides, "free" aglycons, and those conjugates which are below the detection limit in food plants. Extraction efficiencies and HPLC conditions were evaluated and optimized, leading to precision and spiking recovery values of 3–8% and 94–104%, respectively, depending on the analyte. Phytoestrogen levels from more than 40 food items, mostly legumes, were determined using this method. High levels of daidzein and genistein were found in soy products and black beans, whereas sprout items were found to be rich in coumestrol and formononetin.

Keywords: Legumes; soy; isoflavones; phytoestrogens; HPLC

INTRODUCTION

Phytoestrogens include a wide variety of plant products with weak estrogenic activity (Verdeal and Ryan, 1979; Price and Fenwick, 1985) discovered after isoflavones (Shutt et al., 1967) were found to be responsible for the infertility problems of livestock feeding on forage plants such as subterranean clover (Bennets et al., 1946). Since then, more than 300 plants have been reported to cause estrogenic responses in animals (Bradbury and White, 1954; Farnsworth et al., 1975; Shutt, 1976), and many efforts have been undertaken to screen feeds for these agents (Beck, 1964; Pettersson and Kiessling, 1984) to prevent adverse effects of phytoestrogens on the reproductive system of animals. The growing interest in these compounds, particularly isoflavones, is due to recent findings suggesting that these agents might act as cancer-protective agents (Adlercreutz et al., 1993; Coward et al., 1993) as shown in many cell and animal models and properties often connected with cancer prevention such as antioxidant (György et al., 1964; Ikehata et al., 1968; Murakami et al., 1984; Pratt and Birac, 1979; Jha et al., 1985), radical scavenging (Hatano, 1988), hypolipidemic (Mathur et al., 1964; Sharma, 1979), serum cholesterol lowering (Mathur et al., 1964; Sharma, 1979), antiestrogenic (Barnes et al., 1990; Price and Fenwick, 1985; Martin et al., 1978; Verdeal et al., 1980), and antiproliferative effects (Peterson and Barnes, 1991, 1993; Hirano et al., 1989; Fotsis et al., 1993; Schweigerer et al., 1992). In particular, the observed decrease of tumor numbers in vitro and in vivo after treatment with soy products (Barnes et al., 1990, 1994) or after treatment with daidzein (Jing et al., 1993), one of the major isoflavonoid components in soy items, and the suggestive role of soy products in reducing cancer risk (Messina and Barnes,

HPLC has emerged as the method of choice for this task due to its speed, precision, and relatively low cost using reversed-phase C₁₈ stationary matrices, mostly in mixtures of methanol or acetontrile and aqueous acids or buffers as modifiers. Fluorescence detection (Lundh et al., 1988; Wang et al., 1990; Pettersson and Kiessling, 1984; Kitada et al., 1985) and electrochemical detection (Setchell et al., 1987; Kitada et al., 1985, 1986) were shown to be very useful to increase the sensitivity of commonly used UV detection.

Since glycitin and glycitein occur only in trace amounts in soy foods (Murakami et al., 1984; Kudou et al., 1991), most studies have restricted measurements to the predominant analytes daidzein, genistein, and their 7-O-glucosides (Kitada et al., 1985, 1986; Esaki et al., 1990; Matsuura et al., 1989; Murakami et al., 1984). Some studies included coumestrol (Eldridge, 1982; Murphy, 1981, 1982), and very few quantified all of the aforementioned agents (Jones et al., 1989; Setchell et al., 1987). Phytoestrogens occur as glycosides in soy foods (Kudou et al., 1991; Coward et al., 1993), but several authors preferred to measure the total aglycon content including formononetin and biochanin A after hydrolyzing the conjugates to their respective aglycons (Pettersson and Kiessling, 1984; Lundh et al., 1988; Wang et al., 1990; Setchell et al., 1987). Few studies use internal standards to adjust for analyte loss during extraction and separation of phytoestrogens (Eldridge, 1982; Murakami et al., 1984; Jones et al., 1989; Esaki et al., 1990; Wang et al., 1990; Coward et al., 1993). Additionally, the chemical structures of the standards utilized were not related to the analytes and, consequently, the compounds applied in these studies were not well suited as internal standards (Franke et al., 1993).

To our knowledge only one study has analyzed the phytoestrogen content in various foods other than soy items (Jones et al., 1989); however, no detectable levels were reported for the 107 food items analyzed.

We describe a fast, reliable, sensitive, and precise method for the diode array reversed-phase HPLC analysis of the most common isoflavones daidzein, genistein,

¹⁹⁹¹⁾ sparked the efforts in analyzing phytoestrogens in soy products.

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formononetin, and biochanin A (Williams and Harborne, 1989) and of coumestrol, another potent phytoestrogen, after efficient extraction and acid hydrolysis of conjugates. This method was applied to more than 40 widely consumed food items, mostly legumes, since this plant family is known to contain high amounts of these agents (Williams and Harborne, 1989). Flavone, a compound structurally very similar to the analytes, was selected as internal standard among several chemicals tested, and fluorescence detection was used to increase selectivity for the coumestrol analysis. We evaluated the proposed procedure for extraction efficiency, precision, and spiking recovery of phytoestrogens. Additionally we examined the influence of food origin, maturation, and processing, such as boiling and freezing, on phytoestrogen levels and compared the phytoestrogen content of different parts of pods.

EXPERIMENTAL PROCEDURES

Apparatus. HPLC analyses were carried out on a System Gold chromatograph with an autosampler Model 507 and a dual-channel diode array detector Model 168 (all units from Beckman, Fullerton, CA) and a fluorescence detector model FD100 (GTI/SpectroVision; Concord, MA). Absorbance measurements were performed on a DU-62 spectrophotometer (Beckman).

Chemicals. Methanol, hydrochloric acid, acetic acid, 96% ethanol, and dimethyl sulfoxide (DMSO) and all solvents used for HPLC and absorbance readings were of analytical or HPLC grade from Fisher (Fair Lawn, NJ). Butylated hydroxytoluene (BHT), β -glucosidase (from almonds), β -glucuronidase/sulfatase (from $Helix\ pomatia$), sodium acetate, and biochanin A were purchased from Sigma Chemical Co. (St. Louis, MO). Daidzein, formononetin, and genistein were obtained from ICN (Costa Mesa, CA) and flavone from Aldrich (Milwaukee, WI).

Food Items. Soybean seeds 1 grown in the United States (JFC Co.; San Francisco, CA) were purchased from a local supermarket in May 1993 (batch 1) and in January 1994 (batch 2). Soybean seeds 3 and green peas both grown in Japan were from Savings Co., Japan; the former were roasted according to a traditional Japanese recipe by soaking the seeds for 40 min in water followed by draining for 2 h, roasting for 40 min in an open pan, and toasting again in the oven at 180 °C for 40 min. Frozen soybeans from Taiwan (Shirakiku Co., Honolulu, HI; boiled for 12 min) and raw soybeans were purchased from a local Asian food store. Tofu prepared from U.S.-grown soybean seeds with the CaSO₄ coagulation method was obtained from a local manufacturer (Kanai Co., Honolulu, HI). Alfalfa sprouts and radish sprouts were purchased from a local supermarket. Soybean seeds 2 and soy flour were organically grown in the United States (Arrowhead Mill, Hereford, TX) and were obtained from a local health food store together with black bean seeds 1, kidney bean seeds, large lima bean seeds, small lima bean seeds, black-eyed bean seeds, fava bean seeds, small white bean seeds, red bean seeds (boiled for 20 min), pink bean seeds, white navy bean seeds, yellow split peas, broad bean seeds (fried for 7 min), mung bean seeds, green split peas, green peas, round split peas, Chinese peas, lentils, red lentils, urad dahl, masur dahl, kala chana (all from Country Grown Co., CA), organically grown clover sprouts (Aloha Sprout Co., Haleiwa, HI), barley, and sesame. Black bean seeds 2, green split peas, great northern bean seeds, pinto bean seeds, garbanzo bean seeds (all from Golden Grain Co., San Leandro, CA), Chinese peas (boiled for 5 min), and green bean pods (1, raw; 2, boiled for 12 min) were obtained from different local grocery stores.

Standard Solutions, Calibration Curves, and Calculation of Food Levels. Phytoestrogen stock solutions were prepared by dissolving the crystalline standards first in 20 μ L of DMSO followed by addition of 96% ethanol to give 2–5 M solutions. The purity of these solutions was checked by HPLC analysis with monitoring at the individual compound's absorption maximum. The purity (percent) of the standard was

Table 1. HPLC and Calibration Parameters of Phytoestrogens Monitored at 260 nm

| compound | retention time (min) | k' | slope | intercepta | r | concn range (µM) |
|-------------------------|-------------------------|-------|---------|------------|-------|---------------------|
| daidzein | 5.4 | 3.15 | 1.5326 | -0.0088 | 0.996 | 0.7-35.0 |
| genistein | 8.3 | 5.38 | 0.8016 | -0.0127 | 0.998 | 1.2-52.0 |
| coumestrol ^b | 8.8 | 5.77 | 2.3653° | -0.0229 | 0.997 | 0.8-32.0 |
| formononetin | 10.5 | 7.08 | 0.7354 | -0.0181 | 0.995 | 1.2 - 48.0 |
| biochanin A | 12.6 | 8.69 | 1.0507 | -0.0287 | 0.989 | 1.0-50.0 |
| flavone ^d | 14.4 | 10.08 | e | e | e | e |

^a Concentration as a function of peak area units. ^b Responding on fluorometric detection (excitation = 330 nm, emission = 418 nm). ^c Sensitivity higher by a factor of 1.6 when monitored at 342 nm. ^d Internal standard. ^e Not determined.

calculated by dividing the peak area of the compound by all peak areas in the chromatogram and multiplying by 100, assuming that contaminants or byproducts have the same light absorption properties as the standard. Compounds with less than 95% purity were discarded. The concentration of the stock solutions was determined by absorbance readings at the wavelength with maximum absorption (λmax) using molar extinction coefficients (ϵ) (Ollis, 1962) after the stock solutions were diluted to appropriate concentrations with 96% ethanol except for coumestrol, which was diluted with acetonitrile (Wolfbeis and Schaffner, 1980) using the following values: daidzein, $\lambda_{max} = 250 \text{ nm}, \epsilon = 20 893$; genistein, $\lambda_{max} = 263 \text{ nm},$ $\epsilon = 37 \ 154$; formononetin, $\lambda_{\text{max}} = 256 \ \text{nm}$, $\epsilon = 29 \ 512$; biochanin A, $\lambda_{\max} = 263$ nm, $\epsilon = 20$ 893; coumestrol, $\lambda_{\max} = 339$ nm, $\epsilon =$ 22 300. The final stock concentration of each individual standard was calculated using the absorbance reading adjusted for the purity.

Calibration curves were obtained for each standard with high linearity (r > 0.995) by plotting the standard concentration as a function of the peak area obtained from HPLC analyses with 20 μ L injections. For this purpose the stock solutions of the standards were diluted with the mobile phase to nine different concentrations, starting with 25% of the lowest expected concentration and ending with 5 times the highest expected food concentration. Each concentration was analyzed by triplicate injections (Table 1).

Calculation of analytes from food items was performed by using obtained HPLC area units, the slope of the calibration curve, and adjustment for internal standard recovery and thermolability.

Chromatographic Conditions. HPLC analyses were carried out on an Adsorbosphere C_{18} (10 × 4.6 mm i.d.; 5 μ m) direct-connect guard column (Alltech, Deerfield, IL) coupled to a Nova-Pak C_{18} (150 × 3.9 mm i.d.; 4 μ m) reversed-phase column (Waters, Milford, MA). Elution was caried out at a flow rate of 0.8 mL/min with the following solvent system: A = acetonitrile, B = acetic acid/water (10/90 v/v); 23% A in B (v/v) linearly to 70% A in B in 8 min followed by holding at 23% A in B for 12 min, which equilibrates the system for subsequent injections. Analytes were monitored with the dual-channel diode array detector at 260 and 342 nm simultaneously, and peaks were scanned between 190 and 420 nm for identification purposes. The fluorescence detector was used with a 340 nm excitation filter and a 418 nm emission filter.

Extraction and Acid Hydrolysis of Phytoestrogens from Food Items. One gram of powdered dry or freeze-dried food material was finely dispersed in a mixture of 10 mL of 10 M HCl and 40 mL of 96% EtOH (containing 0.05% BHT as antioxidant and 20 ppm of flavone as internal standard) by stirring and sonicating for 10 min followed by refluxing. After 1, 2, 3, and 4 h refluxing periods, the mixture was cooled to room temperature and ethanol lost during the refluxing was replaced; 1.2 mL of this mixture was centrifuged at 850g for at least 10 min, and 20 μ L of clear supernatant was injected directly into the HPLC system.

Extraction and Enzymatic Hydrolysis of Phytoestrogens from Food Items. One gram powdered dry food material was finely dispersed in a mixture of 10 mL of water and 40 mL of 96% EtOH (containing 0.05% BHT as antioxidant and 20 ppm of flavone as internal standard) by stirring and

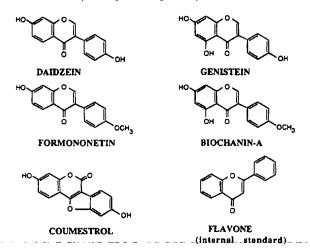


Figure 1. Structures of phytoestrogens analyzed.

sonicating for 10 min followed by refluxing for 3 h. Two milliliters of clear, centrifuged extract was evaporated to dryness under reduced pressure and redissolved in 2.0 mL of 0.1 M acetate buffer (pH 5) containing 2 mg of β -glucosidase and 40 μ L of β -glucuronidase/sulfatase (Setchell et al., 1987); 50 μL of this mixture was used for HPLC analysis of "free" aglycons, and the residual 1.95 mL was incubated for 24 h at 37 °C. After centrifugation, 20 μL of clear supernatant was injected directly into the HPLC system for total phytoestrogen analysis.

RESULTS AND DISCUSSION

HPLC Analysis of Phytoestrogens. Among several HPLC columns tested with authentic phytoestrogen standards (Figure 1), a Nova-Pak C₁₈ column showed the best selectivity, recovery, and peak shape for daidzein, genistein, coumestrol, formononetin, and biochanin A as shown in Figure 2 (trace A) and Table 1. The use of a Supelcosil LC₁₈ column (Supelco, Bellefonte, PA) resulted in poor selectivity and peak shape, and a Spherex 5 C₁₈ column (Phenomenex, Torrence, CA) led to extremely low recoveries, probably by binding the analytes to the stationary phase. An acetonitrile mixture with 10% acetic acid was chosen as mobile phase since other modifiers examined (sodium phosphate buffer, pH 5, 0.1 M hydrochloric acid, trifluoroacetic acid) led in combination with acetonitrile and/or methanol and/or tetrahydrofuran to peak tailing, lower selectivity, and/or lower recovery. A fast and steep linear solvent gradient was applied to elute analytes and internal standard, covering a wide polarity range, within 20 min. The analytes were monitored at or very near their absorption maximum (Table 2) with a dualchannel diode array detector at 260 and 342 nm. Coumestrol was selectively detected at 342 nm as well as by fluorescence detection (see Experimental Procedures for details).

Detection limits (Table 3) obtained from authentic standards were found to be extremely low, even lower than those found for carotenoids (Franke et al., 1993), although the latter agents possess higher extinction coefficients. This might be explained by the much lower background noise observed in the proposed system for phytoestrogens when monitoring takes place at 260 nm compared to the conditions used for the carotenoid analyses. Coumestrol showed a 1.6-fold lower detection limit when monitored at its absorption maximum (342 nm); a further decrease of detection limit is possible by using fluorescence detection at higher pH of the mobile

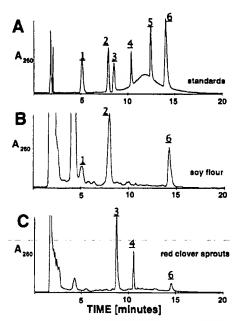


Figure 2. HPLC trace of phytoestrogen standards (A) and extracts from soy flour (B) and red clover sprouts (C) monitored at 260 nm. Peak identification: 1, daidzein; 2, genistein; 3, coumestrol; 4, formononetin; 5, biochanin A; 6, flavone (internal standard). Analyte concentration (mg/L) in trace A: 1, 4.05; 2, 2.38; 3, 3.74; 4, 3.17; 5, 2.42; 6, 9.01. Analyte concentration (mg/L) in trace B: 1, 15.94; 2, 16.80; 6, 16.03. Analyte concentration (mg/L) in trace C: 3, 116.09; 4, 34.45; 6, 17.16.

Table 2. Absorption Maxima of Phytoestrogens Determined with the Proposed Diode Array HPLC Method

| compound | absorption maxima (nm) | |
|--------------|--------------------------|--|
| daidzein | 260 sh, ^a 300 | |
| genistein | 258, 290 sh, 328 | |
| coumestrol | 260 sh, 301, 342 | |
| formononetin | 260 sh. 300 | |
| biochanin A | 266, 332 sh | |
| flavone | 252, 293, 310 sh | |

a sh, shoulder.

Table 3. Detection Limits^a of Phytoestrogens Analyzed with the Proposed HPLC Method

| | • | | ng/g | | |
|--------------|--------------------|------------|-------------|------------|--|
| analyte | nM | ng/mL | ь | c | |
| daidzein | 5.15 | 1.31 | 65.5 | 13.1 | |
| genistein | 8.75 | 2.37 | 118.3 | 23.7 | |
| coumestrol | 25.70 ^d | 6.89^{d} | 344.7^{d} | 68.9^{d} | |
| formononetin | 7.25 | 1.95 | 97.2 | 19.5 | |
| biochanin A | 13.0 | 3.70 | 184.8 | 37.0 | |

^a Determined with a 20 μ L HPLC injection at a signal to noise ratio of 5 and monitoring at 260 nm. b Data calculated for 1 g of food material extracted in 50 mL. CData calculated for 5 g of food material extracted in 50 mL. d Detection limit lower by a factor of 1.6 when monitored at 342 nm.

phase since the maximum 436 nm emission intensity of the coumestrol monoanion occurs at pH 8 (Wolfbeis and Schaffner, 1980).

Calibration curves with extremely high linearity were obtained from all analytes (r > 0.995) in the concentration range expected for food extracts (Table 1).

Extraction Effici ncy and Evaluation of Phytoestrogens Using Soy Flour. Aqueous ethanol (77%) was chosen as extraction solvent since phytoestrogens occur in soybeans originally almost entirely (>95%) as glycosides and malonyl esters (Kudou et al., 1991;

Coward et al., 1993), and consequently polar solvents have been recommended for efficient extraction (Pettersson and Kiessling, 1984; Murphy, 1981; Setchell et al., 1987; Coward et al., 1993). Extraction yields of phytoestrogens from soybeans gave 11% higher values when refluxing was used compared to shaking at room temperature (Kudou et al., 1991), and flavonoids were found to give optimal yields when refluxed in an aqueous polar organic solvent (Keinanen, 1993). Additionally, 80% aqueous ethanol was recommended as solvent system for flavonoid extractions because it gave the best efficiency and safety when refluxing was applied as extraction method (Keinanen, 1993).

Acid hydrolysis (Pettersson and Kiessling, 1984; Wang et al., 1990) converting originally occurring phytoestrogen conjugates (Walz, 1931; Walter, 1941; Kudou et al., 1991) into their respective aglycons was chosen to measure all conjugated and "free" analytes in one step. Consequently, various conjugates that may be below the detection limit add to the amounts of free aglycons after hydrolysis, leading to final amounts more likely to be above the detection limit. Additionally, HPLC is facilitated using hydrolyzed samples due to the reduced number of analytes. Acid conditions may also destroy unwanted coextractives interfering with the detection of analytes and increase recoveries by efficiently destroying proteins bound to analytes.

Extraction efficiencies were optimized by varying refluxing period and hydrochloric acid concentration (Figure 3). Although 1 h of refluxing with 3.0 M HCl gave excellent yields for daidzein and refluxing for 3 h with 1.0 or 1.5 M HCl gave high yields for genistein, only refluxing for 1 and 3 h in the presence of 2 M HCl gave maximum yields for daidzein and genistein, respectively. Refluxing in 77% aqueous ethanol without added acid resulted in 3% yield of free aglycon relative to total aglycon content, in good agreement with earlier reports (Murphy, 1982; Wang et al., 1990; Coward et al., 1993). Enzymatic hydrolysis using the extract obtained with 77% ethanol was found to be slightly less effective than acid hydrolysis (Figure 3) and was abandoned.

These results show that a significantly longer refluxing time in more concentrated acid is required for optimum extraction efficiency of all analytes compared to earlier studies (Wang et al., 1990). The extraction procedure optimized using soy flour was applied for the analysis of all other food items in this study, although it cannot be excluded that items different from the examined soy flour will have different extraction efficiencies. Due to possible variations of extraction efficiencies in different foods, hourly aliquots of all items studied were analyzed during the entire 3 h extraction period and only the highest concentration calculated among the three values is reported in Table 4.

Further purification of extracts by defatting with petroleum (Pettersson and Kiessling, 1984), freezing (Murakami et al., 1984), solid phase extraction (Pettersson and Kiessling, 1984; Setchell et al., 1987), or phase separation (Dziedzic and Dick, 1982; Lane and Newman, 1987) was found to be unnecessary, since interfering compounds were not eliminated by these procedures and the HPLC performance was not negatively influenced, even after injection of approximately 400 crude extracts obtained from food items analyzed in this study. In fact, defatting powdered soy foods with hexane prior to extraction resulted in 30–40% lower yields for daidzein and genistein.

During extraction, the ratio of solvent volume (milliliters) versus food material (grams) was never lower than 10 in the protocol applied, which is the recommended value for exhaustive extractions of isoflavonoids (Coward et al., 1993). Ratios as great as 500 were found to have no influence on extraction efficiencies or reproducibility in this study.

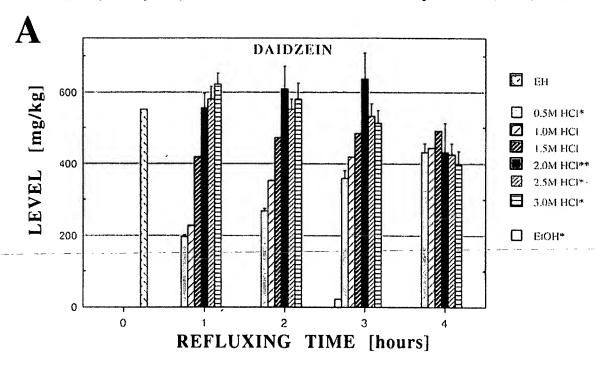
Precision and spiking recoveries listed in Table 5 confirm the validity of the proposed procedure, in particular considering the fact that excellent values for interassay precision were obtained by two different analysts.

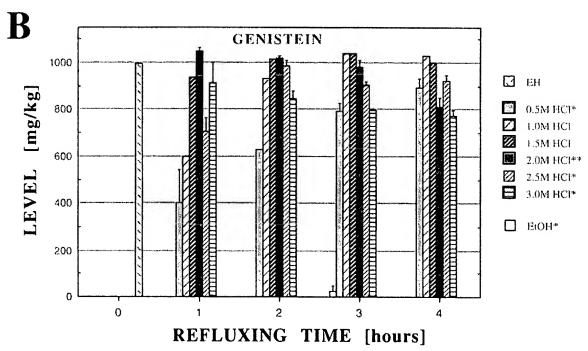
Internal Standard. Internal standards are recommended for analyses aimed at high precision and accuracy to adjust for potential degradation or loss of analytes during the various processes involved in the measurements (Franke et al., 1993). Therefore, we searched for compounds having structures similar to those of the analytes, capable of mimicking the fate of the analytes during extraction and HPLC analysis. Flavone was selected as internal standard among several candidates, such as o-hydroxyacetophenone, o-methoxyacetophenone, propiophenone, n-butyrophenone, and 4-chromanone, due to the structural similarity of this compound to the analytes, its elution in an "empty" and "late" part of the chromatogram, avoiding interference with the analytes, and its stability against heat and acids (Figure 4) applied in the extraction procedure.

Thermostability of Analyzed Phytoestrogens. The stability of the five analytes under the conditions established for food extractions was examined by refluxing authentic standards in 77% ethanol containing 2.0 M hydrochloric acid (Figure 4). Only flavone was found to be entirely stable during refluxing for up to 4 h, while biochanin A and genistein degraded by 5% and 13%, respectively, and the daidzein and formononetin peaks increased by 11% and 14%, respectively, after 3 h of refluxing. Therefore, food levels determined with the proposed procedure were adjusted for these changes.

Identification of Extracted Analytes from Foods after HPLC Separation. All analytes detected by HPLC in food extracts were identified by comparing retention times and UV absorption patterns with authentic standards analyzed in the same batch as the food extracts (Table 2) and by comparing UV absorption data with the data given in the literature (Dewick, 1982; Markham, 1982; Williams and Harborne, 1989). Coumestrol was detected with the 342 nm trace and the trace obtained by fluorescence detection, in addition to the 260 nm trace (see Experimental Procedures for details).

Food Levels. The measured food levels of total daidzein, genistein, coumestrol, formononetin, and biochanin A are listed in Table 4 as means of two to six separate analyses. Coefficients of variation between measurements were found to be between 3% and 11%. In general, soy foods and black beans were found to have very high levels of total daidzein and genistein, ranging from 0.3% to 1.4% relative to dry weight. Sprout items, especially clover sprouts, showed extremely high concentrations of total coumestrol and formononetin. Most food items showed little or none of the compounds analyzed in this study, confirming earlier results when none of the 107 examined food items showed any detectable phytoestrogen levels (Jones et al., 1989). Boiling foods did not seem to destroy daidzein or genistein significantly as shown by results obtained





- mean and standard deviation of duplicate analysis
- " mean and standard deviation of quadruplicate analysis

Figure 3. Extraction efficiency of daidzein (A) and genistein (B) from soy flour depending on hydrochloric acid concentration varying from 0.5 to 3.0 M in 77% aqueous ethanol and refluxing time. Extracting with 2 M HCl gave maximum yields for both daidzein and genistein with refluxing times of 3 h and 1 h, respectively. Refluxing with 77% ethanol (no acid present) ("EtOH") resulted in 3% yield relative to maximum yields for both analytes. Enzymatic hydrolysis ("EH") with β -glucosidase and β -glucuronidase/sulfatase of the ethanol extract resulted in 87% and 95% yield compared to the maximum yield for daidzein and genistein, respectively.

from black beans, but results from soybeans indicated that roasting causes losses of 15% and 21% for daidzein and genistein, respectively. These losses are probably due to the preparative step prior to the roasting process in which the seeds are soaked and drained, thereby being partly extracted by water (Wang et al., 1990) as

opposed to the heat exposure (Coward et al., 1993). The method of production of tofu did not affect the isoflavone levels, as noted earlier (Esaki et al., 1990; Coward et al., 1993), since the dry weight level of tofu was found to be very similar to the one from soybean seeds. Soybean seeds grown in Japan versus those grown in

Table 4. Total Phytoestrogen Levels^a of Analyzed Food Items

| | | | mg/kg of food material | | | |
|---------------------------------|----------|-----------|------------------------|--------------|-----------------------|--|
| food item ^b | daidzein | genistein | coumestrol | formononetin | biochanin A | |
| soybean seeds 1, dry (batch1) | 1001.3 | 1022.7 | ndc | nd | nd | |
| soybean seeds 1, dry (batch2) | 676.4 | 940.2 | nd | nd | nd | |
| soybean seeds 2, dry | 700.6 | 1082.0 | nd | nd | nd | |
| soybean seeds 3, dry | 1006.5 | 1382.4 | nd | nd | \mathbf{nd} | |
| soybean seeds 3, roasted | 848.1 | 1105.5 | nd | nd | nd | |
| soybean seeds 4, fresh, raw | 90.0 | 91.7 | nd | nd | nd | |
| freeze-dried (64.3% water loss) | 252.0 | 257.0 | nd | nd | nd | |
| soybean seeds 5, fresh, boiled | 68.5 | 69.4 | nd | nd | nd | |
| freeze-dried (69.5% water loss) | 224.7 | 227.4 | nd | nd | \overline{nd} | |
| soybean seeds 6, fresh, frozen | 282.1 | 315.4 | nd | nd | nd | |
| freeze-dried (61.8% water loss) | 738.5 | 825.7 | \mathbf{nd} | nd | nd | |
| soybean hulls 6, fresh, frozen | nd | 18.4 | \mathbf{nd} | nd | nd | |
| freeze-dried (75.2% water loss) | nd | 74.1 | nd | nd | nd | |
| soy flour | 654.7 | 1122.6 | nd | nd | nd | |
| tofu | 113.4 | 166.4 | nd | nd | nd | |
| freeze-dried (86.5% water loss) | 840.2 | 1232.7 | nd | nd | nd | |
| black bean seeds 1, dry | 698.5 | 612.2 | nd | nd | nd | |
| black bean seeds 2, boiled | 269.5 | 277.1 | nd ` | nd | nd | |
| freeze-dried (65.2% water loss) | 774.4 | 796.4 | nd | nd | nd | |
| green beans 1, fresh, raw | nd | nd | nd | 1.5 | tr^d | |
| freeze-dried (93.0% water loss) | nd | nd | nd | 21.1 | tr | |
| green beans 2, fresh, boiled | nd | nd | nd | tr | tr | |
| freeze-dried (93.7% water loss) | nd | nd | nd | tr | tr | |
| large lima bean seeds, dry, raw | nd | nd | 14.8 | tr | nd | |
| large lima beans seeds, boiled | nd | nd | nd | 0.1 | nd | |
| freeze-dried (93.7% water loss) | nd | nd | nd | 0.2 | nd | |
| red bean seeds, dry | nd | 3.1 | tr | nd | nd | |
| garbanzo bean seeds, dry | nd | nd | nd | nd | 15.2 | |
| kidney bean seeds, cooked | nd | nd | nd | nd | 4.1 | |
| freeze-dried (68.6% water loss) | nd | nd | nd | nd | 13.2 | |
| pinto bean seeds, dry | nd | nd | 36.1 | tr | 5.6 | |
| white navy bean seeds, dry | nd | nd | nd | nd | tr | |
| small lima bean seeds, dry | nd | nd | nd | 5.5 | 3.7 | |
| great northern bean seeds, dry | nd | nd | nd | nd | 6.0 | |
| broad bean seeds, fried | nd nd | 12.9 | nd | 2.1 | nd | |
| pink bean seeds, dry | nd | nd | nd | 10.5 | nd nd | |
| black-eyed bean seeds, dry | nd | nd | nd | nd | 17.3 | |
| small white bean seeds, dry | nd | 7.4 | nd | 8.2 | nd | |
| yellow split peas, dry | nd nd | nd | nd | nd | 8.6 | |
| green split peas, dry | 72.6 | nd | nd | tr | nd | |
| round split peas, dry | nd | nd nd | | | | |
| chinese peas, boiled | nd | nd nd | 81.1 | nd a | nd | |
| freeze-dried (90.2% water loss) | nd nd | nd nd | nd 1 | nd | 93.1 | |
| | | _ | nd | nd | 10.1 | |
| kala chana seeds, dry | nd nd | 6.4 | 61.3 | nd | 12.6 | |
| mung bean seeds, dry | | nd | nd | 6.1 | nd | |
| mung bean sprouts | nd | nd | nd | tr | nd | |
| freeze-dried (92.9% water loss) | nd | nd | nd | tr | nd | |
| clover sprouts | nd | 3.5 | 280.6 | 22.8 | 4.4 | |
| freeze-dried (95.0% water loss) | nd | 69.4 | 5611.4 | 456.5 | 88.1 | |
| alfalfa sprouts | nd | nd | 46.8 | 3.4 | nd | |
| freeze-dried (93.5% water loss) | nd | nd | 720.1 | 51.7 | nd | |

none of these phytoestrogens were found in the following: green peas, fava beans, Japanese; green peas, red beans boiled; lentils; red lentils; urad dahl; masur dahl; radish sprouts; barley; and sesame

^a Means of repeated analyses (two to six times) from dry or freeze-dried item with relative standard deviations between 3% and 11%. ^b Food items with different numbers derived from different sources; beans refer to entire fruit (pod) including hulls and seeds: soybean seeds 1, grown in U.S. from JFC Co.; soybean seeds 2, organically grown in U.S. from Arrowhead Mills Co.; soybean seeds 3, grown in Japan from Savings Co.; soybeans 4, fresh from local market; soybeans 5, fresh from local market; soybeans 6, frozen from Taiwan; tofu from U.S.-grown soybean seeds; soy flour, from organically grown seeds in U.S. (Arrowhead Mills); black beans 1, from Country Grown Co.; black beans 2, from Golden Grain Co.; green beans 1 and 2, from various local stores. ^c nd, not detected. ^d tr, trace (between 60% and 100% of detection limit given in Table 3).

the United States showed roughly the same daidzein levels but were 27% higher in genistein levels. Compared to dry raw soybean seeds, frozen soybean seeds obtained from fresh pods were 20-30% lower in daidzein and genistein; raw soybean seeds from pods stored at room temperature were found to be 75% lower in these analytes. These differences are probably due to the maturation stage since phytoestrogen levels increase

with germination (Wong et al., 1965) or maturation of seeds (Kudou et al., 1991) and are most likely not due to the storage temperature since the analytes were shown to be relatively stable against heat (Figure 4). In soybean hulls only 20% of the seed's usual genistein level was found and daidzein was not detected at all. Soybeans grown organically in the United States showed no significant difference in levels for genistein compared

Table 5. Precision and Spiking Recoveries Obtained with the Proposed Method for Phytoestrogen Analysis from Soy Flour

| | • | precision (n = | 6) | | | | | |
|--------------|-----------------|------------------------------|------------------|---------------|--------------------------|--------------------|-------------------------|--|
| | | coefficient of variation (%) | | | spiking recovery $(n=4)$ | | | |
| compound | mean (mg/kg) | within assay | between assay | μg present | μg spiked | recovery (mean) | RSD ^a (%) | |
| daidzein | 654.7 | 2.7 | 8.2 | 35.3 | 44.8 | 104.7 | 5.1 | |
| genistein | 1122.6 | 2.4 | 3.8 | 58.8 | 40.5 | 93.7 | 4.6 | |
| coumestrol | ь | ь | ь | ь | 47.5 | 94.0 | 4.8 | |
| formononetin | ь | Ь | ь | ь | 51.3 | 98.0 | 1.1 | |
| biochanin A | Ь | ь | Ь | ь | 30.7 | 101.1 | 2.5 | |

^a Relative standard deviation. ^b Not present.

Table 6. Comparison of Total Daidzein and Genistein Levels Obtained by the Proposed Method and Previous Studies

| food item | food source | daidzein (mg/kg) | genistein (mg/kg) | study |
|---------------------|-------------------|------------------|-------------------|-----------------------------------|
| soy flour | USA | 655 | 1123 | present study |
| | USA | 658-742 | 837-939 | Coward et al. (1993) ^b |
| soybean | USA | 676-1001 | 940-1082 | present study d |
| • | Japan | 1007 | 1382 | present study |
| | Asia | 574 | 935 | Coward et al. (1993) |
| | USA | 2060 | 2040 | Kudou et al. (1991) |
| | USA | 341 | 430 | Wang et al. (1990) |
| | USA | 754 | 1181 | Matsuura et al. (1989) |
| | Europe | 706 | 1000 | Pettersson and Kiessling (1984 |
| | USA | 206-548 | 457-1402 | Eldridge and Kwolek (1983)d |
| | USA | 22-72 | 507-664 | Murphy (1982)* |
| | USA | 256 | 956 | Pratt and Birac (1979) |
| textured soy | USA | 568 | 568 | Setchell et al. (1987) |
| soy flake | USA | 221 | 280 | Setchell et al. (1987) |
| defatted flakes | USA (Maple Arrow) | 1165 | 1951 | Kitada et al. (1986) |
| defatted flakes | USA | 419 | 1411 | See and Morr (1984) |
| defatted flakes | USA | 721 | 1222 | Eldridge and Kwolek (1983) |
| tofu# | | | | |
| (87% water loss) | USA | 840 | 1233 | present study |
| (80-87% water loss) | Asia | 438-1036 | 910-1420 | Coward et al. (1993) ⁴ |

^a Daidzin and genistin levels were converted to daidzein and genistein levels and added to reported aglycon concentrations to give total daidzein and genistein levels. ^b Four brands analyzed. Mean levels (rel standard deviation): DE = 707 mg/kg (5.1%), GE = 891 mg/kg (4.9%). ^c Three different sources analyzed. Mean levels (rel standard deviation): DE = 793 mg/kg (22.8%), GE = 1015 mg/kg (7.0%). ^d Eight varieties analyzed. Mean levels (rel standard deviation): DE = 447 mg/kg (42.1%), GE = 878 mg/kg (39.2%). ^e Two varieties analyzed. Mean levels (rel standard deviation): DE = 47 mg/kg (75.2%), GE = 586 mg/kg (19.0%). ^f Two or three replicates. Mean levels given in table; rel standard deviation: DE = 13.6%, GE = 4.4%. ^e Levels of freeze-dried material. ^h Two brands analyzed. Mean levels (rel standard deviation): DE = 737 mg/kg (57.4%), GE = 1165 mg/kg (31.0%).

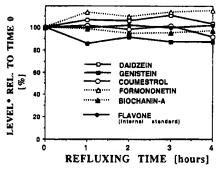


Figure 4. Stability of standards after refluxing with 2 M HCl in 77% aqueous ethanol. Note that only flavone is stable after heating in 77% ethanol/2 M HCl. Biochanin A and genistein degrade by 5% and 13%, respectively, and the daidzein and formononetin peaks increased by 11% and 14%, respectively, requiring adjustment for these changes in food level determinations with this extraction procedure.

to "normally" grown U.S. soybeans but showed 36% lower levels for daidzein. Milling did not affect daidzein or genistein levels since the results of this study showed similar levels for soy flour and organically grown soybeans which both originated from the same source according to the supplier (Arrowhead Mills). A decrease of phytoestrogens during the milling process through the loss of cotyledones or hypocotyls, both shown to

differ greatly in isoflavone accumulation (Kudou et al., 1991; Eldridge and Kwolek, 1983), obviously did not occur in this case.

In conclusion, our proposed procedure represents a fast, easy, reliable, reproducible, and sensitive method requiring little technician time to quantitate total daidzein, genistein, coumestrol, formononetin, and biochanin A levels in food items. Up to 10 food items can be analyzed in duplicate per day from one analyst including extraction, HPLC analysis, and data calculation. The presented values for soy flour and soybean seeds compare very favorably with those published recently (Matsuura et al., 1989; Coward et al., 1993; Dwyer et al., 1994) when based on total daidzein and genistein level (Table 6). Differences in total daidzein and genistein levels of soy items comparing our results with other studies (Murphy et al., 1982; Kudou et al., 1991; Table 6) might be due to differences in the analytical procedure. More likely, however, these differences are due to the different origin of the analyzed foods since plant variety, location, harvesting year, and maturity are known to affect isoflavone levels in soybeans (Eldridge and Kwolek, 1983). This is also indicated by variations in levels found in the same laboratory as a function of food origin and food batch (Table 6).

The results presented in this study covering the most likely phytoestrogenic food sources show a wide range of phytoestrogen types and levels, depending on plant species, plant part, maturation, growing conditions, and processing. These data will be very useful in planning epidemiologic trials aimed at evaluating the potential cancer-protective properties of these agents, since exposure data will be available for foods consumed by the study population. However, the effects of origin, i.e., location, growing conditions, and age of food plants, and the known phytoalexin properties of isoflavonoids (Smith and Banks, 1986; Dorr and Guest, 1987) influencing phytoestrogen accumulation must be considered to obtain correct exposure data using published food levels of these agents.

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Antioxidant Capacity of Tea and Common Vegetables

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Previously, some fruits were shown to contain high antioxidant activities. In this paper, we report the antioxidant activities of 22 common vegetables, one green tea, and one black tea measured using the automated oxygen radical absorbance capacity assay with three different reactive species: a peroxyl radical generator, a hydroxyl radical generator, and Cu^{2+} , a transition metal. Based on the fresh weight of the vegetable, garlic had the highest antioxidant activity (μ mol of Trolox equiv/g) against peroxyl radicals (19.4) followed by kale (17.7), spinach (12.6), Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, eggplant (9.8–3.9), cauliflower, potato, sweet potato, cabbage, leaf lettuce, string bean, carrot, yellow squash, iceberg lettuce, celery, and cucumber (3.8–0.5); kale had the highest antioxidant activity against hydroxyl radicals followed by Brussels sprouts, alfalfa sprouts, beets, spinach, broccoli flowers, and the others. The green and black teas had much higher antioxidant activities against peroxyl radicals than all these vegetables. However, the tea also showed a prooxidant activity in the presence of Cu^{2+} , which was not found with any of the vegetables studied.

Keywords: Antioxidant; free radical; tea; vegetable

INTRODUCTION

Consumption of fruits and vegetables has been associated with lower incidence and lower mortality rates of cancer in several human cohort and case-control studies for all common cancer sites (Ames et al., 1993; Doll, 1990; Dragsted et al., 1993; Willett, 1994a). The antitumorigenic effects of vegetables were also found in experiments using cells (Maeda et al., 1992) and animals (Belman, 1983; Bingham, 1990; Bresnick et al., 1990; Maltzman et al., 1989; Stoewsand et al., 1988; Stoewsand et al., 1989; Wattenberg and Coccia, 1991). There is a highly significant negative association between intake of total fruits and vegetables and cardioand cerebrovascular disease mortality (Acheson and Williams, 1983; Armstrong et al., 1975; Burr and Sweetnam, 1982; Phillips et al., 1978; Verlangieri et al., 1985). Vegetarians and nonvegetarians with a high intake of fruits and vegetables also have reduced blood pressure (Ascherio et al., 1992; Sacks and Kass, 1988).

The protection that fruits and vegetables provide against diseases, including cancer and cardio- and cerebrovascular diseases, has been attributed to the various antioxidants, especially antioxidant vitamins, including ascorbic acid and α -tocopherol, contained in these fruits and vegetables (Ames, 1983; Gey, 1990; Gey et al., 1991; Riemersma et al., 1989; Stähelin et al., 1991a,b; Steinberg et al., 1989, 1991; Willett, 1994b). However, the majority of the antioxidant activity of a fruit or vegetable may be from compounds other than vitamin C, vitamin E, or β -carotene. For example, some flavonoids that are often found in the human diet have antioxidant activities (Bors and Saran, 1987; Bors et al., 1990; Hanasaki et al., 1994). Our laboratory has already reported that some common fruits have high

antioxidant activities which cannot be accounted for by their vitamin C content (Wang et al., 1996). We also found that some flavonoids had much stronger antioxidant activities against peroxyl radicals than vitamin E, vitamin C, and glutathione (Cao et al., in press). The objective of this study was to determine the antioxidant capacities of 22 common vegetables, one green tea, and one black tea by using the oxygen radical absorbance capacity (ORAC) assay (Cao et al., 1993, 1995). Three different reactive species were used in the ORAC assay: (i) 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), a peroxyl radical (ROO*) generator, (ii) $Cu^{2+}H_2O_2$, mainly a hydroxyl radical (OH*) generator, and (iii) Cu^{2+} , a transition metal.

MATERIALS AND METHODS

Chemicals. β -Phycoerythrin (β -PE) from *Porphydium cruentum* was purchased from Sigma (St. Louis, MO). The β -PE that was used in these experiments usually lost more than 90% of its fluorescence within 30 min in the presence of 4 mmol/L AAPH. AAPH was purchased from Wako Chemicals USA Inc. (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI).

Tea and Vegetables. Twenty-two vegetables were purchased on three separate occasions from local supermarkets. The 22 vegetables were garlic, kale, spinach, Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, eggplant, cauliflower, potato, sweet potato, cabbage, leaf lettuce, string bean, carrot, yellow squash, iceberg lettuce, celery, and cucumber. The green tea used in the study was Chin Chu oriental blend tea. The black tea (all black teas are fermented teas) was a dried powder and provided by Tea Trade Health Research Association.

Sample Preparation. The black tea was completely dissolved in deionized water (5 mg/mL) and used for ORAC assay directly after suitable dilution with phosphate buffer (75 mM, pH 7.0). The green tea was brewed for 30 min in deionized water (1:60, w/v, 95-100 °C). The edible portion of a vegetable was weighed and then homogenized by using a blender after adding deionized water (1:2, w/v). The brewed green tea and vegetable homogenate were then centrifuged

already reported that some common fruits have high

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Table 1. Total Antioxidant Capacity of Tea and Common Vegetables^a

| | dry matter ORAC _{ROO} | | CROO.b | ORA | AC _{OH} , ⁶ | OR. | AC _{Cu} ¢ | antioxidant |
|------------------|--------------------------------|----------------|--------------|---------------|---------------------------------|----------------|--------------------|--------------------|
| item | (%) | WM basis | DM basis | WM basis | DM basis | WM basis | DM basis | score ^d |
| green tea | | - | 814 ± 30 | - | 35.8 ± 6.0 | | -41.9 ± 7.1 | |
| black tea | | | 927 | | NM ^e | | NM | |
| garlic | 42.9 ± 2.7 | 19.4 ± 3.1 | 46 ± 9 | 1.1 ± 0.4 | 2.7 ± 0.9 | 2.7 ± 0.41 | 6.4 ± 1.1 | 23.2 |
| kale | 10.4 ± 1.7 | 17.7 ± 0.6 | 179 ± 32 | 6.2 ± 0.3 | 61.3 ± 7.5 | 0.2 ± 0.03 | 2.3 ± 0.5 | 24.1 |
| spinach | 9.8 ± 0.6 | 12.6 ± 0.3 | 129 ± 6 | 2.8 ± 0.4 | 29.6 ± 6.1 | 1.6 ± 0.19 | 16.0 ± 1.9 | 17.0 |
| Brussels sprouts | 14.0 ± 0.5 | 9.8 ± 1.8 | 70 ± 10 | 5.4 ± 0.8 | 38.5 ± 4.7 | 0.6 ± 0.09 | 4.3 ± 0.9 | 15.8 |
| alfalfa sprouts | 8.0 ± 0.2 | 9.3 ± 0.7 | 117 ± 12 | 4.6 ± 0.5 | 58.1 ± 6.9 | 0.6 ± 0.05 | 7.0 ± 0.7 | 14.5 |
| broccoli flowers | 15.1 ± 0.3 | 8.9 ± 1.0 | 59 ± 5 | 2.4 ± 0.3 | 15.6 ± 1.8 | 1.6 ± 0.09 | 10.5 ± 0.4 | 12.9 |
| beets | 12.0 ± 2.7 | 8.4 ± 0.2 | 81 ± 28 | 3.1 ± 0.1 | 36.0 ± 7.7 | 0.2 ± 0.03 | 2.2 ± 0.7 | 11.7 |
| red bell pepper | 9.8 ± 0.5 | 7.1 ± 0.5 | 74 ± 9 | 0.6 ± 0.1 | 6.2 ± 0.9 | 0.4 ± 0.08 | 3.7 ± 0.7 | 8.1 |
| onion | 11.2 ± 0.7 | 4.5 ± 0.5 | 40 ± 2 | 0.5 ± 0.1 | 4.1 ± 0.9 | 0.6 ± 0.17 | 5.4 ± 1.4 | 5.6 |
| corn | 18.6 ± 2.4 | 4.0 ± 0.5 | 22 ± 4 | 2.2 ± 0.2 | 11.7 ± 0.5 | 1.0 ± 0.15 | 5.2 ± 0.7 | 7.2 |
| eggplant | 5.3 ± 1.1 | 3.9 ± 0.3 | 80 ± 22 | 1.1 ± 0.1 | 22.4 ± 3.5 | 0.1 ± 0.03 | 1.3 ± 0.2 | 5.1 |
| cauliflower | 8.3 ± 0.9 | 3.8 ± 1.0 | 46 ± 11 | 1.1 ± 0.1 | 13.6 ± 2.3 | 0.2 ± 0.07 | 2.7 ± 0.6 | 5.1 |
| potato | 22.7 ± 2.1 | 3.1 ± 1.0 | 15 ± 5 | 1.0 ± 0.2 | 4.4 ± 1.2 | 0.5 ± 0.11 | 2.3 ± 0.5 | 4.6 |
| sweet potato | 21.8 ± 1.7 | 3.0 ± 0.3 | 14 ± 2 | 1.0 ± 0.1 | 4.4 ± 0.3 | 0.3 ± 0.03 | 1.2 ± 0.2 | 4.3 |
| cabbage | 9.5 ± 0.7 | 3.0 ± 0.3 | 32 ± 2 | 1.5 ± 0.1 | 15.8 ± 0.5 | 0.3 ± 0.02 | 3.4 ± 0.4 | 4.8 |
| leaf lettuce | 5.4 ± 0.5 | 2.6 ± 0.2 | 49 ± 7 | 1.4 ± 0.2 | 25.0 ± 1.4 | 0.1 ± 0.03 | 1.5 ± 0.4 | 4.1 |
| string bean | 7.4 ± 1.5 | 2.0 ± 0.5 | 30 ± 8 | 1.7 ± 0.2 | 24.2 ± 3.3 | 0.2 ± 0.04 | 2.3 ± 0.6 | 3.9 |
| carrot | 7.7 ± 0.6 | 2.1 ± 0.7 | 26 ± 8 | 0.8 ± 0.1 | 10.3 ± 0.4 | 0.5 ± 0.06 | 7.2 ± 1.0 | 3.4 |
| yellow squash | 12.0 ± 3.1 | 1.5 ± 0.3 | 17 ± 3 | 1.1 ± 0.2 | 12.5 ± 1.5 | 0.2 ± 0.02 | 1.7 ± 0.2 | 2.8 |
| iceberg lettuce | 3.7 ± 1.2 | 1.2 ± 0.2 | 39 ± 12 | 0.7 ± 0.1 | 23.2 ± 6.9 | 0.4 ± 0.08 | 11.9 ± 3.2 | 2.3 |
| celery | 5.0 ± 0.4 | 0.6 ± 0.1 | 13 ± 2 | 0.3 ± 0.1 | 6.0 ± 1.0 | 0.2 ± 0.09 | 4.3 ± 2.0 | 1.1 |
| cucumber | 3.5 ± 0.2 | 0.5 ± 0.1 | 15 ± 2 | 0.3 ± 0.1 | 7.1 ± 1.4 | 0.3 ± 0.02 | 9.2 ± 0.8 | 1.1 |

^a Data expressed as means \pm SEM of three samples purchased and analyzed independently, except for the black tea. ^b Data expressed as μ mol of Trolox equiv/g of wet matter (WM) or dry matter (DM). ^c Data expressed as $\times 10^3$ units/g of wet matter (WM) or dry matter (DM). ^d Antioxidant score = ORAC_{ROO'} + ORAC_{OH'} + ORAC_{Cu} (WM basis). ^e NM, not measured.

at 34000g for 30 min (4 °C). The supernatant (water soluble fraction) was:recovered and used directly for the ORAC assay after suitable dilution with the phosphate buffer. The pulp (water insoluble fraction) was washed twice with deionized water and further extracted by using pure acetone (1:4, w/v) with shaking at room temperature for 30 min. Acetone has been used by our laboratory (Wang et al., 1996) and others (Daniel et al., 1989; Mass et al., 1991) to extract antioxidants from fruit pulp. The acetone extract was recovered after centrifugation (34000g, 10 min, 4 °C), and the sample was used for the ORAC assay after suitable dilution with phosphate buffer. The ORAC activity of a vegetable or the green tea was calculated by adding the ORAC activity from its water soluble fraction and its pulp fraction extracted with acetone. The dry matter of a vegetable was determined after drying the vegetable at 40 °C for 1 week.

Automated ORAC Assay. The automated ORAC assay was carried out on a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ) with fluorescent filters (ex. 540 nm; em. 565 nm). The procedure was based on a previous report of Cao and coworkers (Cao et al., 1993), as modified for the COBAS FARA II (Cao et al., 1995). Briefly, in the final assay mixture (0.4 mL total volume), β -PE (16.7 nM) was used as a target of free radical (or oxidant) attack, with either (i) AAPH (4 mM) as a peroxyl radical generator (ORAC_{ROO} assay), (ii) H₂Q₂-Cu²⁺ $[H_2Q_{\phi'},0.3\%;Cu^{2^+}$ (as CuSO₄). $9\,\mu\rm M]$ as mainly a hydroxy radical generator (ORAC_{OH} assay), or (iii) Cu²⁺ (as CuSO₄) (18 μM) as a transition metal oxidant (ORAC_{Cu} assay). Trolox was used as a control standard. A 0.1 mM stock solution was stable for at least 1 month at $-80\,^{\circ}$ C. The analyzer was programmed to record the fluorescence of β -PE every 2 min after AAPH. H₂O₂-Cu²⁺, or Cu²⁺ was added. All fluorescent measurements were expressed relative to the initial reading. Final results were calculated using the differences of areas under the β -PE decay curves between the blank and a sample and are expressed as μ mol of Trolox equiv/g of tea or vegetables (Cao et al., 1993, 1995), except when Cu^{2+} alone (i.e., without H_2O_2) was used as an oxidant in the assay. In the presence of Cu2+ alone, Trolox cannot be used as an antioxidant standard since Trolox may act as a prooxidant in the presence of Cu²⁺ (Cao and Cutler, 1993). Therefore, the result of the ORACcu assay in this case was calculated using (areasample - areablank)/areablank and expressed as antioxidant units; I unit equals the antioxidant activity which increases the area under the β -PE decay

curve by 100% in the $ORAC_{Cu}$ assay. A negative $ORAC_{Cu}$ value indicated a Cu^{2+} -initiated prooxidant activity.

RESULTS

The antioxidant activities against peroxyl radicals (ORAC_{ROO} activity) of 22 common vegetables, one green tea, and one black tea are shown in Table 1. Based on the *fresh* or *wet* weight of a vegetable, garlic and kale were in the top quintile of ORACROO measured in the 22 vegetables. Spinach, Brussels sprouts, alfalfa sprouts, broccoli flowers, beets, red bell pepper, onion, corn, and eggplant had ORAC_{ROO} values that fell in the middle three quintiles (3.9-12.6). Cauliflower, potato, sweet potato, cabbage, leaf lettuce, string beans, carrot, yellow squash, iceberg lettuce, celery, and cucumber were in the lowest quintile of ORAC_{ROO} activities of the vegetables measured. However, based on the dry weight of a vegetable, kale had the highest ORACROO activity followed by spinach, alfalfa sprouts, beets, eggplant, red bell pepper, Brussels sprouts, broccoli flowers, leaf lettuce, garlic, cauliflower, onion, and iceberg lettuce. Cabbage, string beans, carrots, corn, yellow squash, cucumber, potato, sweet potato, and celery were in the lowest quintile (below 32.0) of ORAC_{ROO} activities expressed on a dry matter basis. Green and black teas had much higher ORACROS activities than any of the vegetables studied (4.5-5-fold higher than kale and 60-70-fold higher than celery, based on the dry weight).

The antioxidant activities against hydroxyl radicals. (ORAC_{OH} activity) of the vegetables and green tea are also shown in Table 1. Based on the *fresh* or *wet* weight of a vegetable, kale had the highest ORAC_{OH} activity followed by Brussels sprouts, alfalfa sprouts, beets, spinach, broccoli flowers, corn, string beans, cabbage, leaf lettuce, eggplant, cauliflower, yellow squash, garlic, potato, sweet potato, carrot, iceberg lettuce, red bell pepper, onion, celery, and cucumber. Based on the *dry* weight of a vegetable, kale also had the highest ORAC_{OH} activity followed by alfalfa sprouts, Brussels sprouts, beets, spinach, leaf lettuce, string bean, iceberg

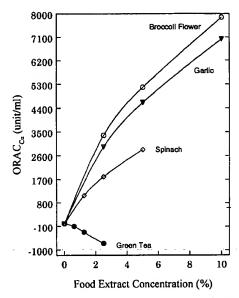


Figure 1. Antioxidant/prooxidant activities of green tea, broccoli flower, garlic, and spinach as a function of their extract concentrations (% of the undiluted extracts). The positive $ORAC_{Cu}$ values indicate antioxidant activities, while the negative $ORAC_{Cu}$ values indicate prooxidant activities (see Materials and Methods).

lettuce, eggplant, cabbage, broccoli flower, cauliflower, yellow squash, corn, carrot, cucumber, red bell pepper, celery, potato, sweet potato, onion, and garlic. The ORAC_{OH} activity of green tea, based on *dry* weight, was between that of beets and spinach.

Green tea showed a prooxidant activity (negative $ORAC_{Cu}$ activity) in the presence of Cu^{2+} (without H_2O_2) (Table 1). This Cu^{2+} -initiated prooxidant activity, however, was not found in any vegetables evaluated in this study. Based on the fresh or wet weight of the vegetable, garlic had the highest antioxidant activities against Cu^{2+} (ORAC $_{Cu}$ activity) followed by broccoli flowers, spinach, and the others. However, spinach had the highest ORAC $_{Cu}$ activity, if activity was based on the dry weight, followed by iceberg lettuce, broccoli flowers, and the others.

The 'antioxidant score' of a vegetable shown in Table 1 was calculated by simply adding ORAC_{ROO} (μmol of Trolox equiv), ORACOH (µmol of Trolox equiv), and ORAC_{Cu} (10³ units), based on the wet weight of the vegetable. One nanomole of Trolox equivalent calculated from ORAC_{ROO} assay and 1 ORAC_{Cu} unit calculated from ORAC_{Cu} assay represent a similar area difference under the β -PE decay curve between the blank and a sample, which was used in the ORAC quantification. Because ORAC_{ROO} activity of a vegetable weights the score more heavily than ORACOH or ORACcu activity of the vegetable in the scoring system, the 'antioxidant score' did not rank the vegetables in a significantly different order than what was observed with the ORAC_{ROO} assay. The 'antioxidant score' was not given for the teas since they are dry, not fresh.

The $ORAC_{Cu}$ activities of tea and vegetables were determined using different extract concentrations, since the Cu^{2+} -initiated prooxidant activity of some antioxidants is seen only at a relatively high concentration (Cao and Cutler, 1993). The results in Figure 1 show that in the presence of Cu^{2+} (without H_2O_2), tea acts as a prooxidant at all concentrations, and the *prooxidant* activity increased with increased tea concentration. However, of the tested vegetables including spinach,

garlic, and broccoli flowers, all act as antioxidants against Cu^{2+} , and their *antioxidant* activity increased as their concentration increased in the assay system.

Figure 2 presents the calculated $ORAC_{ROO}$ intake based upon a common measured size or serving. For many of the vegetables this common measured proportion represents a $^{1}/_{2}$ cup serving size except for garlic (1 clove), onion (1 tablespoon), potato (1 potato), and lettuce (1 leaf). In Figure 2, the common serving size is presented in grams. Based upon this calculation, kale, beets, red bell pepper, Brussels sprouts, broccoli flowers, spinach, potatoes, and corn likely provide a significant amount of $ORAC_{ROO}$ in the diet if these vegetables are consumed on a regular basis. Frequency of consumption of the individual vegetables would be the other factor determining which vegetables contribute the most to the ORAC consumed in a common diet.

DISCUSSION

The ORAC assay developed recently by Cao and coworkers (Cao et al., 1993, 1995) provides a unique and novel way to evaluate the potential antioxidant activities of various compounds and biological samples. This method is superior to other similar methods for two reasons. First, the ORAC assay system uses an areaunder-curve (AUC) technique and thus combines both inhibition time and inhibition degree of free radical action by an antioxidant into a single quantity (Cao et al., 1995). Other similar methods (Ghiselli et al., 1994; Glazer, 1990; Miller et al., 1993; Wayner et al., 1985; Whitehead et al., 1992) use either the inhibition time at a fixed inhibition degree or the inhibition degree at a fixed time as the basis for quantitating the results. Second, different free radical generators or oxidants can be used in the ORAC assay. This is important because the measured antioxidant activity of a biological sample depends upon which free radical or oxidant is used in the assay (Cao et al., 1996a,b).

Peroxyl radical (ROO*) is a common free radical found in the body and used in the antioxidant activity assays (Wayner et al., 1985; Glazer, 1990; Cao et al., 1993, 1995; Ghiselli et al., 1994). It is slightly less reactive than OH* and thus possesses an "extended " half-life of seconds instead of nanoseconds (Grisham, 1992). The total antioxidant capacity of some common fruits was thus determined by us using the ORAC_{ROO*} assay (Wang et al., 1996), which measures all *traditional* antioxidants including ascorbic acid, α -tocopherol, β -carotene, glutathione, bilirubin, uric acid, melatonin (Cao et al., 1993; Pieri et al., 1994), and flavonoids (Cao et al., in press).

In the current study, Cu²⁺-H₂O₂ (a "OH•" generator) and Cu2+ alone were also used to assess the antioxidant activities of one green tea and 22 vegetables. Most of the "OH" thought to be generated in vivo comes from metal-dependent reduction of H2O2, except during abnormal exposure to ionizing radiation. In vitro the metal can be titanium, copper, iron, or cobalt, but the best candidates for promoters of OH formation in vivo seem to be iron and, to a smaller extent, copper. Cu^{2+} H₂O₂ or Cu²⁺ alone is frequently used in inducing oxidative damage to protein and nucleic acids (Parthasarathy et al., 1989; Sato et al., 1992). The ORACOH assay with Cu²⁺-H₂O₂ as a OH generator measures compounds like mannitol, glucose, uric acid (at physiological concentrations), proteins, and transition metal chelators, but not compounds, such as ascorbic acid, that react directly with copper and produce reactive species.

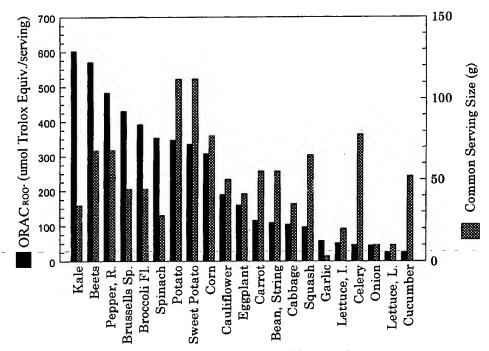


Figure 2. Amount of $ORAC_{ROO'}$ activity consumed (μ mol of Trolox equiv) (left y axis) per common serving or measured quantity (g) (right y axis). Common serving sizes were obtained from USDA Agriculture Handbook No. 8-11 (*Composition of Foods: Vegetables and Vegetable Products*).

The $ORAC_{Cu}$ assay using copper alone measures not only the antioxidant activity (positive $ORAC_{Cu}$ value) of a compound which can sequester transition metals but also the transition metal-initiated *prooxidant* activity (negative $ORAC_{Cu}$ value) of a compound, such as ascorbic acid (Cao and Cutler, 1993) and some flavonoids (Cao et al., in press).

At this point, we do not have a good indication as to which radical generator provides the 'best' estimate of antioxidant activity of the vegetables. Perhaps, the formulation of an 'antioxidant score', which takes into account the antioxidant activities determined by the three different reactive species or oxidants, can give us some additional useful information. We calculated the 'antioxidant score' of each vegetable in this study by simply adding ORAC_{ROO}, ORAC_{OH}, and ORAC_{Cu} values, based on wet weight of the vegetable, since 1 nmol of Trolox equiv calculated from ORACROO assay and 1 ORAC_{Cu} unit calculated from ORAC_{Cu} assay represent a similar area difference under the $\beta\text{-PE}$ decay curve between the blank and a sample. The ORAC_{ROO} value of a vegetable weights the score more heavily than the ORAC_{OH} or ORAC_{Cu} value of the vegetable in the scoring system, which also seems reasonable because peroxyl radicals tend to be more prevalent in biological systems. However, the 'antioxidant score' did not rank the vegetables in a significantly different order than what was observed with the ORACROO assay.

Our results demonstrated clearly that all vegetables tested in this study had antioxidant activities against not only peroxyl radicals but also hydroxyl radicals and transition metals (Cu^{2+}), although their $ORAC_{ROO}$, $ORAC_{OH}$, and $ORAC_{Cu}$ activities vary considerably from one kind of vegetable to another. It is an important finding that these vegetables (and also fruits like strawberry, unpublished data) acted as antioxidants when a transition metal oxidant was used in the ORAC assay and the antioxidant activity also increased as their concentrations increased. The transition metal-initiated prooxidant actions of ascorbic acid (Beach and

Giroux, 1992) and α -tocopherol (Iwatsuki et al., 1995; Maiorino et al., 1993; Yoshida et al., 1994) have been described. Using Cu^{2+} - H_2O_2 in the ORAC assay, it was also found that ascorbic acid and Trolox (at a relatively high concentration), a water soluble α -tocopherol analogue, acted as prooxidants (Cao and Cutler, 1993). Therefore, in terms of the antioxidant quality in vitro, the natural antioxidant mixture contained in fruits or vegetables appears to be better than a single antioxidant or a simple antioxidant mixture of ascorbic acid, α -tocopherol, and β -carotene.

The antioxidant capacity varies considerably from one kind of vegetable to another, similar to what we found in fruits (Wang et al., 1996). The ORAC_{ROO} activities of kale and spinach were similar to that observed in strawberries (Wang et al., 1996) whether the data were based on wet or dry weight. For example, based on the wet weight of a fresh vegetable, the ORACROO activity (which measures all traditional antioxidants) for kale was about 2 times the activity measured in beet and broccoli flowers, 8-9 times the activity measured in carrots and string beans, and 29-35 times the activity measured in celery and cucumber. Based upon a common measured or serving size, kale, beets, red peppers, Brussels sprouts, broccoli flowers, spinach, potatoes, and corn likely provide the largest amount of ORACROO consumed from vegetables (Figure 2), although frequency of consumption of the individual vegetables would be another factor determining which vegetables contribute the most to ORAC consumed in a common diet.

The green and black teas had much higher antioxidant activities against peroxyl radicals than all fruits and vegetables that we have examined. Their ORAC-ROO activity, based on dry weight, was 4.5-6.0 times the activity measured in kale and strawberry (Wang et al., 1996). The ORACOH activity of the green tea, based on the dry weight, was only 58% of that measured in kale. The ORACOH activity of the tea was actually compromised by the Cu^{2+} used in the assay, since the

ORAC_{Cu} activity of the tea was negative, indicating a prooxidant activity of the tea in the presence of Cu²⁺. It seems clear that some tea components can absorb hydroxyl radicals and other reactive species produced from the reaction between Cu²⁺ and H₂O₂. Some tea components apparently can produce reactive species through direct reactions among these tea components, Cu²⁺, and O₂, when Cu²⁺-H₂O₂ is used as a reactive species generator in the ORAC assay. It is also possible that some tea components, such as some flavonoids (Cao et al., in press), can play these two opposite roles at the same time. However, the transition metal-initiated prooxidant actions of tea, ascorbic acid, and α -tocopherol may not be important in vivo, where transition metals will be largely sequestered, except perhaps in certain diseases involving metal overload. Recent experiments have already demonstrated the inhibition by tea and tea polyphenols of tumorigenesis in different animal models (Yang and Wang, 1993), although the effect of tea consumption on cancer risk in humans as revealed by epidemiologic studies is less clear (International Agency for Research on Cancer, 1991).

The antioxidant defense system of the body is composed of different antioxidant components. The antioxidant capacities of these antioxidant components depend upon which free radicals or oxidants are produced in the body. Some fruits and vegetables contain a group of natural antioxidants that have not only a high antioxidant activity but also a good antioxidant quality. Therefore, the supplementation of these natural antioxidants through a balanced diet containing enough fruits and vegetables could be much more effective and economical than the supplementation of an individual antioxidant, such as ascorbic acid or α -tocopherol, in protecting the body against various oxidative stresses.

In summary, the antioxidant activities of 22 common vegetables, one green tea, and one black tea were measured using the automated ORAC assay with three different reactive species: a peroxyl radical generator, a hydroxyl radical generator, and Cu2+, a transition metal. Based on the fresh weight of a vegetable, garlic had the highest antioxidant activity against peroxyl radicals followed by kale, spinach, Brussels sprouts, alfalfa sprouts, and others, while kale had the highest antioxidant activity against hydroxyl radicals followed by Brussels sprouts, alfalfa sprouts, beets, spinach, and the others. Kale also had the highest ORAC_{ROO} activity, when results were expressed on a dry weight basis. The green and black teas had much higher antioxidant activity against peroxyl radicals than all of the vegetables tested in this study. However, the tea exhibited a prooxidant activity in the presence of Cu²⁺, which has also been reported for the antioxidants, ascorbic acid and α-tocopherol; this prooxidant activity was not found in the vegetables analyzed in this study. Therefore, the supplementation of natural antioxidants through a balanced diet containing enough fruits and vegetables could be the most effective in protecting the body against various oxidative stressors.

ABBREVIATIONS USED

AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; ORAC, oxygen radical absorbance capacity; ORAC_{ROO'}, peroxyl radical absorbance capacity; ORAC_{OH'}, hydroxyl radical absorbance capacity; ORAC_{Cu}, antioxidant capacity against Cu^{2+} ; β -PE, β -phycoerythrin;

Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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JF9602535

[®] Abstract published in Advance ACS Abstracts, October 1, 1996.

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L7 2518 TROLOX

=> s vegetable protein

L8 10374 VEGETABLE PROTEIN

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L13
=> s 113 and 17
             5 L13 AND L7
L14
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L14 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2002 ACS - --- --
     2002:283086 CAPLUS
ΑN
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     136:368667
     A comparative study on the various in vitro assays of active oxygen
ΤI
     scavenging activity in foods
     Murakami, M.; Yamaguchi, T.; Takamura, H.; Matoba, T.
ΑU
     Graduate School of Human Culture, Nara Women's Univ., Nara, 630-8506,
CS
     Journal of Food Science (2002), 67(2), 539-541
SO
     CODEN: JFDSAZ; ISSN: 0022-1147
     Institute of Food Technologists
PB
     Journal
DT
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LA
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RE.CNT 14
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L14
     2001:494886 CAPLUS
ΑN
     135:210250
DN
     Effect of Processing and Storage on the Antioxidant Ellagic Acid
TΤ
     Derivatives and Flavonoids of Red Raspberry (Rubus idaeus) Jams
     Zafrilla, Pilar; Ferreres, Federico; Tomas-Barberan, Francisco A.
ΑU
     Laboratorio de Fitoquimica Department of Food Science and Technology,
CS
     CEBAS (CSIC), Murcia, 30080, Spain
     Journal of Agricultural and Food Chemistry (2001), 49(8), 3651-3655
SO
     CODEN: JAFCAU; ISSN: 0021-8561
PB
     American Chemical Society
DT
     Journal
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L14
     1999:229219 CAPLUS
ΑN
     131:31293
DN
     Assessment of the Antioxidant Potential of Scotch Whiskeys by Electron
ΤI
     Spin Resonance Spectroscopy: Relationship to Hydroxyl-Containing Aromatic
     Components
     McPhail, Donald B.; Gardner, Peter T.; Duthie, Garry G.; Steele, Gordon
ΑU
     M.; Reid, Kenneth
CS
     Rowett Research Institute, Aberdeen, AB21 9SB, UK
     Journal of Agricultural and Food Chemistry (1999), 47(5), 1937-1941
SO
     CODEN: JAFCAU; ISSN: 0021-8561
     American Chemical Society
PΒ
DT
     Journal
     English
              THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
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- L14 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2002 ACS
- AN 1998:797800 CAPLUS
- DN 130:119566
- TI Antioxidant efficacy of phytoestrogens in chemical and biological model systems
- AU Mitchell, Julie H.; Gardner, Peter T.; McPhail, Donald B.; Morrice, Philip C.; Collins, Andrew R.; Duthie, Garry G.
- CS Division of Micronutrients and Lipid Metabolism, Rowett Research Institute, Aberdeen, AB21 9SB, UK
- SO Archives of Biochemistry and Biophysics (1998), 360(1), 142-148 CODEN: ABBIA4; ISSN: 0003-9861
- PB Academic Press
- DT Journal
- LA English
- L14 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
- AN 1999:55802 BIOSIS
- DN PREV199900055802
- TI Antioxidant efficacy of phytoestrogens in chemical and biological model systems.
- AU Mitchell, Julie H. (1); Gardner, Peter T.; McPhail, Donald B.; Morrice, Philip C.; Collins, Andrew R.; Duthie, Garry G.
- CS (1) Div. Micronutrients Lipid Metabolism, Rowett Res. Inst., Bucksburn, Aberdeen AB21 9SB UK
- SO Archives of Biochemistry and Biophysics, (Dec. 1, 1998) Vol. 360, No. 1, pp. 142-148.
 ISSN: 0003-9861.
- DT Article
- LA English

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- L13 ANSWER 1 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:898782 CAPLUS
- TI Oxygen Radical Absorbing Capacity of **Phenolics** in Blueberries, Cranberries, Chokeberries, and Lingonberries
- AU Zheng, Wei; Wang, Shiow Y.
- CS Fruit Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD, 20705, USA
- SO Journal of Agricultural and Food Chemistry ACS ASAP CODEN: JAFCAU; ISSN: 0021-8561
- PB American Chemical Society

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DT Journal
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LA English

RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT

- L13 ANSWER 2 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:862335 CAPLUS
- TI Black tea represents a major source of dietary **phenolics** among regular tea drinkers
- AU Rechner, A. R.; Wagner, E.; Van Buren, L.; Van De Put, F.; Wiseman, S.; Rice-Evans, C. A.
- CS Centre for Age-Related Diseases, Guy's, King's and St Thomas's School of Biomedical Sciences, King's College London, London, SE1 9RT, UK

- SO Free Radical Research (2002), 36(10), 1127-1135 CODEN: FRARER; ISSN: 1071-5762
- PB Taylor & Francis Ltd.
- DT Journal
- LA English
- RE.CNT 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 3 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:720110 CAPLUS
- DN 137:324488
- TI Nutritional Value of Cherry Tomatoes (Lycopersicon esculentum Cv. Naomi F1) Harvested at Different Ripening Stages
- AU Raffo, Antonio; Leonardi, Cherubino; Fogliano, Vincenzo; Ambrosino, Patrizia; Salucci, Monica; Gennaro, Laura; Bugianesi, Rossana; Giuffrida, Francesco; Quaglia, Giovanni
- CS Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, Rome, 00178, Italy
- SO Journal of Agricultural and Food Chemistry (2002), 50(22), 6550-6556 CODEN: JAFCAU; ISSN: 0021-8561
- PB American Chemical Society
- DT Journal
- LA English
- RE.CNT 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 4 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:679979 CAPLUS
- DN 137:337094
- TI Identification and Quantification of Antioxidant Components of Honeys from Various Floral Sources
- AU Gheldof, Nele; Wang, Xiao-Hong; Engeseth, Nicki J.
- CS Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL, 61801, USA
- SO Journal of Agricultural and Food Chemistry (2002), 50(21), 5870-5877 CODEN: JAFCAU; ISSN: 0021-8561
- PB American Chemical Society
- DT Journal
- LA English
- RE.CNT 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 5 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:675452 CAPLUS
- DN 137:351770
- TI Induction of Antioxidant Flavonol Biosynthesis in Fresh-Cut Potatoes. Effect of Domestic Cooking
- AU Tudela, Juan A.; Cantos, Emma; Espin, Juan C.; Tomas-Barberan, Francisco A.; Gil, Maria I.
- CS Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS-CSIC, Murcia, 30080,

Spain Journal of Agricultural and Food Chemistry (2002), 50(21), 5925-5931 SO CODEN: JAFCAU; ISSN: 0021-8561 PB American Chemical Society DΤ Journal English LA THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD RE.CNT 31 ALL CITATIONS AVAILABLE IN THE RE FORMAT L13 ANSWER 6 OF 97 CAPLUS COPYRIGHT 2002 ACS 2002:669882 CAPLUS ΑN 137:351874 DN Extraction of Anthocyanins and Other Phenolics from Black ΤI Currants with Sulfured Water ΑU Cacace, J. E.; Mazza, G. Food Research Program Pacific Agri-Food Research Centre, Agriculture and CS Agri-Food Canada, Summerland, BC, R3T 2N2, Can. Journal of Agricultural and Food Chemistry (2002), 50(21), 5939-5946 SO CODEN: JAFCAU; ISSN: 0021-8561 American Chemical Society DT Journal LA English THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD RE.CNT 38 ALL CITATIONS AVAILABLE IN THE RE FORMAT L13 ANSWER 7 OF 97 CAPLUS COPYRIGHT 2002 ACS ΑN 2002:556539 CAPLUS 137:200567 DN Effect of Freezing and Storage on the Phenolics, Ellagitannins, Flavonoids, and Antioxidant Capacity of Red Raspberries Mullen, William; Stewart, Amanda J.; Lean, Michael E. J.; Gardner, Peter; ΑU Duthie, Garry G.; Crozier, Alan Plant Products and Human Nutrition Group Graham Kerr Building Division of CS Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences University of Glasgow, Glasgow, G12 8QQ, UK Journal of Agricultural and Food Chemistry (2002), 50(18), 5197-5201 SO CODEN: JAFCAU; ISSN: 0021-8561 American Chemical Society PB DT Journal English LA THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD RE.CNT 32 ALL CITATIONS AVAILABLE IN THE RE FORMAT L13 ANSWER 8 OF 97 CAPLUS COPYRIGHT 2002 ACS AΝ 2002:556538 CAPLUS 137:246675 DN Ellagitannins, Flavonoids, and Other Phenolics in Red ΤI Raspberries and Their Contribution to Antioxidant Capacity and Vasorelaxation Properties Mullen, William; McGinn, Jennifer; Lean, Michael E. J.; MacLean, Margaret ΑU R.; Gardner, Peter; Duthie, Garry G.; Yokota, Takoa; Crozier, Alan Plant Products and Human Nutrition Group, Division of Biochemistry and CS Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK Journal of Agricultural and Food Chemistry (2002), 50(18), 5191-5196 SO CODEN: JAFCAU; ISSN: 0021-8561 PΒ American Chemical Society DT Journal LA English

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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L13 ANSWER 9 OF 97 CAPLUS COPYRIGHT 2002 ACS AN 2002:294369 CAPLUS

RE.CNT 25

- DN 137:28188
- TI Can Apple Antioxidants Inhibit Tumor Cell Proliferation?

 Generation of H2O2 during Interaction of Phenolic Compounds with Cell
 Culture Media
- AU Lapidot, Tair; Walker, Michael D.; Kanner, Joseph
- CS Department of Food Science, ARO Volcani Center, Bet Dagan, 50250, Israel
- SO Journal of Agricultural and Food Chemistry (2002), 50(11), 3156-3160 CODEN: JAFCAU; ISSN: 0021-8561
- PB American Chemical Society
- DT Journal
- LA English
- RE.CNT 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 10 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:283086 CAPLUS
- DN 136:368667
- TI A comparative study on the various in vitro assays of active oxygen scavenging activity in foods
- AU Murakami, M.; Yamaguchi, T.; Takamura, H.; Matoba, T.
- CS Graduate School of Human Culture, Nara Women's Univ., Nara, 630-8506, Japan
- SO Journal of Food Science (2002), 67(2), 539-541 CODEN: JFDSAZ; ISSN: 0022-1147
- PB Institute of Food Technologists
- DT Journal
- LA English
- RE.CNT 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 11 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:207082 CAPLUS
- DN 136:308940
- TI Influence of Cultivar on Quality Parameters and Chemical Composition of Strawberry Fruits Grown in Brazil
- AU Cordenunsi, Beatriz Rosana; Oliveira do Nascimento, Joao Roberto; Genovese, Maria Ines; Lajolo, Franco Maria
- CS Laboratorio de Quimica, Bioquimica e Biologia Molecular de Alimentos, Departamento de Alimentos e Nutricao Experimental, FCF, Universidade de Sao Paulo, Sao Paulo, SP, 05508-900, Brazil
- SO Journal of Agricultural and Food Chemistry (2002), 50(9), 2581-2586 CODEN: JAFCAU; ISSN: 0021-8561
- PB American Chemical Society
- DT Journal
- LA English
- RE.CNT 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 12 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:124129 CAPLUS
- DN 136:215800
- TI Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum)
- AU Martinez-Valverde, Isabel; Periago, Maria J.; Provan, Gordon; Chesson, Andrew
- CS Food Science Department, Veterinary Faculty, University of Murcia, Murcia, E-30071, Spain
- SO Journal of the Science of Food and Agriculture (2002), 82(3), 323-330 CODEN: JSFAAE; ISSN: 0022-5142
- PB John Wiley & Sons Ltd.
- DT Journal
- LA English
- RE.CNT 54 THERE ARE 54 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT

- L13 ANSWER 13 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2002:86249 CAPLUS
- DN 136:276072
- TI Composition of a chemopreventive proanthocyanidin-rich fraction from cranberry fruits responsible for the inhibition of 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced ornithine decarboxylase (ODC) activity
- AU Kandil, Fayez E.; Smith, Mary Ann L.; Rogers, Randy B.; Pepin, Marie-France; Song, Lynda L.; Pezzuto, John M.; Seigler, David S.
- CS Department of Plant Biology and Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL, 61801, USA
- SO Journal of Agricultural and Food Chemistry (2002), 50(5), 1063-1069 CODEN: JAFCAU; ISSN: 0021-8561
- PB American Chemical Society
- DT Journal
- LA English
- RE.CNT 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 14 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2001:907199 CAPLUS
- DN 136:117658
- TI Effect of Principal Polyphenolic Components in Relation to Antioxidant Characteristics of Aged Red Wines
- AU Arnous, Anis; Makris, Dimitris P.; Kefalas, Panagiotis
- CS Department of Food Quality Management and Laboratory of Chemistry of Natural Products, Mediterranean Agronomic Institute of Chania (MAICh), Chania, 73100, Greece
- SO Journal of Agricultural and Food Chemistry (2001), 49(12), 5736-5742 CODEN: JAFCAU; ISSN: 0021-8561
- PB American Chemical Society
- DT Journal
- LA English
- RE.CNT 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 15 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2001:787653 CAPLUS
- DN 136:85043
- TI Extraction of **Phenolics** and Changes in Antioxidant Activity of Red Wines during Vinification
- AU Burns, Jennifer; Gardner, Peter T.; Matthews, David; Duthie, Garry G.; Lean, Michael E. J.; Crozier, Alan
- CS Plant Products and Human Nutrition Group Division of Biochemistry and Molecular Biology, IBLS University of Glasgow, Glasgow, G12 8QQ, UK
- SO Journal of Agricultural and Food Chemistry (2001), 49(12), 5797-5808 CODEN: JAFCAU: ISSN: 0021-8561
- PB American Chemical Society
- DT Journal
- LA English
- RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 16 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 2001:780236 CAPLUS
- DN 136:363242
- TI Prediction of the antioxidant activity of natural **phenolics** from electrooptical state indices
- AU Dorman, H. J. Damien; Peltoketo, Anna; Huuskonen, Jarmo; Hiltunen, Raimo
- CS Division of Pharmacognosy, University of Helsinki, Helsinki, FIN-00014, Finland
- SO Special Publication Royal Society of Chemistry (2001), 269(Biologically-Active Phytochemicals in Food), 322-324 CODEN: SROCDO; ISSN: 0260-6291

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PB
     Royal Society of Chemistry
DT
     Journal
LA
     English
              THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE.CNT 5
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 17 OF 97 CAPLUS COPYRIGHT 2002 ACS
     2001:771309 CAPLUS
AN
     136:98647
DN
ΤI
     High-throughput fluorescence screening of antioxidative capacity in human
ΑU
     Mayer, Birgit; Schumacher, Martin; Brandstaetter, Helga; Wagner, Franz S.;
     Hermetter, Albin
CS
     Department of Biochemistry, Technische Universitaet Graz, Graz, A-8010,
     Austria
SO
     Analytical Biochemistry (2001), 297(2), 144-153
     CODEN: ANBCA2; ISSN: 0003-2697
PB-
     Academic Press
DT
     Journal
     English
LA
RE.CNT 39
              THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 18 OF 97 CAPLUS COPYRIGHT 2002 ACS
     2001:499677 CAPLUS
AN
     135:256610
DN
TΙ
     Alcoholic beverages and Mediterranean diet in human health. Wine
     phenolics and ethyl alcohol as antioxidants and
     scavengers of oxygen free radicals. Toxicological implications for
     moderate and high alcohol consumption
ΑU
     Valavanidis, Athanasios; Zonaras, Vasilios; Theodoropoulou, Sofia
CS
     Department of Chemistry, University of Athens, Athens, 15771, Greece
SO
     Epitheorese Klinikes Farmakologias kai Farmakokinetikes, International
     Edition (2001), 15(2), 85-96
     CODEN: EFKEEB; ISSN: 1011-6583
PΒ
     Pharmakon-Press
DT
     Journal
     English
LA
RE.CNT 71
             THERE ARE 71 CITED REFERENCES AVAILABLE FOR THIS RECORD
             ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 19 OF 97 CAPLUS COPYRIGHT 2002 ACS
ΑN
     2001:494886 CAPLUS
DN
     135:210250
TΙ
     Effect of Processing and Storage on the Antioxidant Ellagic Acid
     Derivatives and Flavonoids of Red Raspberry (Rubus idaeus) Jams
ΑU
     Zafrilla, Pilar; Ferreres, Federico; Tomas-Barberan, Francisco A.
     Laboratorio de Fitoquimica Department of Food Science and Technology,
CS
     CEBAS (CSIC), Murcia, 30080, Spain
     Journal of Agricultural and Food Chemistry (2001), 49(8), 3651-3655
SO
     CODEN: JAFCAU; ISSN: 0021-8561
PΒ
    American Chemical Society
DT
    Journal
    English
LA
RE.CNT 20
             THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD
             ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 20 OF 97 CAPLUS COPYRIGHT 2002 ACS
    2001:242522 CAPLUS
ΑN
DN
    135:45339
TΙ
    Separation and determination of flavonoids and other phenolic compounds in
    cranberry juice by high-performance liquid chromatography
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Department of Chemistry and Biochemistry, University of Massachusetts,

Chen, H.; Zuo, Y.; Deng, Y.

AU CS

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Dartmouth, North Dartmouth, MA, 02747, USA
     Journal of Chromatography, A (2001), 913(1-2), 387-395
SO
     CODEN: JCRAEY; ISSN: 0021-9673
     Elsevier Science B.V.
PB
DT
     Journal
     English
LA
              THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE.CNT 21
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 21 OF 97 CAPLUS COPYRIGHT 2002 ACS
     2001:230858 CAPLUS
ΑN
     134:310445
DN
     Low inhibitory activities of food phenolics against binding of
TΤ
     estradiol to human estrogen receptor .alpha.
ΑU
     Mi, Hongbin; Hiramoto, Kazuyuki; Kikugawa, Kiyomi
     School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo,
CS
     192-0392, Japan
     Journal of Oleo Science (2001), 50(4), 255-257
SO
     CODEN: JOSOAP; ISSN: 1345-8957
     Japan Oil Chemists' Society
     Journal
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LA
     English
              THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE.CNT 18
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 22 OF 97 CAPLUS COPYRIGHT 2002 ACS
ΑN
     2001:168507 CAPLUS
DN
     134:352408
     A Cyclic Voltammetry Method Suitable for Characterizing Antioxidant
     Properties of Wine and Wine Phenolics
ΑU
     Kilmartin, Paul A.; Zou, Honglei; Waterhouse, Andrew L.
     Department of Chemistry, The University of Auckland, Auckland, N. Z.
CS
     Journal of Agricultural and Food Chemistry (2001), 49(4), 1957-1965
SO
     CODEN: JAFCAU; ISSN: 0021-8561
     American Chemical Society
PB
DT
     Journal
     English
LA
RE.CNT 30
              THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 23 OF 97 CAPLUS COPYRIGHT 2002 ACS
     2001:90181 CAPLUS
ΑN
     134:256968
DN
     LC coupled to ion-trap MS for the rapid screening and detection of
TΤ
     polyphenol antioxidants from Helichrysum stoechas
     Carini, Marina; Aldini, Giancarlo; Furlanetto, Sandra; Stefani, Rita;
ΑU
     Facino, Roberto Maffei
     Istituto Chimico Farmaceutico Tossicologico, Milan, 42-20131, Italy
CS
     Journal of Pharmaceutical and Biomedical Analysis (2001), 24(3), 517-526
SO
     CODEN: JPBADA; ISSN: 0731-7085
PB
     Elsevier Science B.V.
DT
     Journal
LA
     English
RE.CNT 20
              THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 24 OF 97 CAPLUS COPYRIGHT 2002 ACS
ΑN
     2000:884508 CAPLUS
DN
     134:192729
ΤI
     A Reevaluation of the Peroxynitrite Scavenging Activity of Some Dietary
     Phenolics
     Ketsawatsakul, Uraiwan; Whiteman, Matthew; Halliwell, Barry
AU
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International Antioxidant Research Centre, GKT School of Biomedical

CS

Sciences, London, SE1 9RT, UK

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SO
     Biochemical and Biophysical Research Communications (2000), 279(2),
     692-699
     CODEN: BBRCA9; ISSN: 0006-291X
PB
     Academic Press
DT
     Journal
LA
     English
RE.CNT 78
              THERE ARE 78 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 25 OF 97 CAPLUS COPYRIGHT 2002 ACS
     2000:697789 CAPLUS
ΑN
     134:41428
DN
ΤI
     Effect of skin contact on the antioxidant phenolics in white
ΑU
     Darias-Martin, J. J.; Rodriguez, O.; Diaz, E.; Lamuela-Raventos, R. M.
     Centro Superior de Ciencias Agrarias, Tecnologia de Alimentos, Universidad
CS
     de La Laguna, La Laguna, Tenerife, 38200, Spain
SO T
    Food Chemistry (2000), 71(4), 483-487
     CODEN: FOCHDJ; ISSN: 0308-8146
PΒ
     Elsevier Science Ltd.
DT
     Journal
LA
     English
RE.CNT 27
              THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 26 OF 97 CAPLUS COPYRIGHT 2002 ACS
ΑN
     2000:298202 CAPLUS
DN
     133:57929
     Antioxidant activity of nontocopherol hazelnut (Corylus spp.)
ΑU
     Yurttas, H. C.; Schafer, H. W.; Warthesen, J. J.
CS
     Department of Food Science and Nutrition, University of Minnesota, St.
     Paul, MN, 55108, USA
SO
     Journal of Food Science (2000), 65(2), 276-280
     CODEN: JFDSAZ; ISSN: 0022-1147
     Institute of Food Technologists
PB
DΤ
     Journal
    English
LA
RE.CNT 29
              THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD
             ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 27 OF 97 CAPLUS COPYRIGHT 2002 ACS
     2000:931 CAPLUS
AN
     132:165380
DN
TΤ
     Relationship among Antioxidant Activity, Vasodilation Capacity, and
     Phenolic Content of Red Wines
     Burns, Jennifer; Gardner, Peter T.; O'Neil, Jennifer; Crawford, Sharon;
ΑU
    Morecroft, Ian; McPhail, Donald B.; Lister, Carolyn; Matthews, David;
    MacLean, Margaret R.; Lean, Michael E. J.; Duthie, Garry G.; Crozier, Alan
     Division of Biochemistry and Molecular Biology Institute of Biomedical and
CS
    Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK
     Journal of Agricultural and Food Chemistry (2000), 48(2), 220-230
SO
     CODEN: JAFCAU; ISSN: 0021-8561
PΒ
    American Chemical Society
DT
    Journal
LA
    English
             THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE.CNT 49
             ALL CITATIONS AVAILABLE IN THE RE FORMAT
L13 ANSWER 28 OF 97 CAPLUS COPYRIGHT 2002 ACS
AN
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    Comparative contents of some phenolics in beer, red and white
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- CS Department of Pharmaceutical Chemistry, School of Pharmacy, The Hebrew University-Hadassah Medical School, Jerusalem, 91120, Israel
- SO Nutrition Research (New York) (1999), Volume Date 2000, 20(1), 131-139 CODEN: NTRSDC; ISSN: 0271-5317
- PB Elsevier Science Inc.
- DT Journal
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- RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 29 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1999:750802 CAPLUS
- DN 132:18581
- TI **Phenolics:** blocking agents for heterocyclic amine-induced carcinogenesis
- AU Hirose, M.; Takahashi, S.; Ogawa, K.; Futakuchi, M.; Shirai, T.
- CS First Department of Pathology, Medical School, Nagoya City University, Nagoya, 467-8601, Japan
- SO Food and Chemical Toxicology (1999), 37(9/10), 985-992 CODEN: FCTOD7; ISSN: 0278-6915
- PB Elsevier Science Ltd.
- DT Journal
- LA English
- RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 30 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1999:730762 CAPLUS
- DN 132:236166
- TI Screening of selected flavonoids and phenolic acids in 19 berries
- AU Hakkinen, S.; Heinonen, M.; Karenlampi, S.; Mykkanen, H.; Ruuskanen, J.; Torronen, R.
- CS Department of Clinical Nutrition, University of Kuopio, Kuopio, FIN-70211, Finland
- SO Food Research International (1999), 32(5), 345-353 CODEN: FORIEU; ISSN: 0963-9969
- PB Elsevier Science Ltd.
- DT Journal
- LA English
- RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 31 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1999:377961 CAPLUS
- DN 131:285598
- TI Phenolics and lipid-soluble antioxidants in fruit cuticle of apples and their antioxidant activities in model systems
- AU Ju, Zhiguo; Bramlage, William J.
- CS Department of Plant and Soil Sciences, University of Massachusetts, Amherst, MA, USA
- SO Postharvest Biology and Technology (1999), 16(2), 107-118 CODEN: PBTEED; ISSN: 0925-5214
- PB Elsevier Science Ireland Ltd.
- DT Journal
- LA English
- RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 32 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1999:229219 CAPLUS
- DN 131:31293
- TI Assessment of the Antioxidant Potential of Scotch Whiskeys by Electron Spin Resonance Spectroscopy: Relationship to Hydroxyl-Containing Aromatic

- Components
- AU McPhail, Donald B.; Gardner, Peter T.; Duthie, Garry G.; Steele, Gordon M.; Reid, Kenneth
- CS Rowett Research Institute, Aberdeen, AB21 9SB, UK
- SO Journal of Agricultural and Food Chemistry (1999), 47(5), 1937-1941 CODEN: JAFCAU; ISSN: 0021-8561
- PB American Chemical Society
- DT Journal
- LA English
- RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 33 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1999:68865 CAPLUS
- DN 130:247000
- TI Antioxidant activities of six natural **phenolics** against lipid oxidation induced by Fe2+ or ultraviolet light
- AU Chen, Xiaoying; Ahn, Dong U.
- CS Department of Animal Science, Iowa State University, Ames, IA, 50011-3150, USA
- SO Journal of the American Oil Chemists' Society (1998), 75(12), 1717-1721 CODEN: JAOCA7; ISSN: 0003-021X
- PB AOCS Press
- DT Journal
- LA English
- RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 34 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1999:9530 CAPLUS
- DN 130:236777
- TI Detecting and measuring bioavailability of **phenolics** and flavonoids in humans: pharmacokinetics of urinary excretion of dietary ferulic acid
- AU Bourne, Louise C.; Rice-Evans, Catherine A.
- CS International Antioxidant Research Centre, UMDS-Guy's Hospital, London, SE1 9RT, UK
- SO Methods in Enzymology (1999), 299(Oxidants and Antioxidants, Part A), 91-106
 CODEN: MENZAU; ISSN: 0076-6879
- PB Academic Press
- DT Journal; General Review
- LA English
- RE.CNT 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 35 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1998:797800 CAPLUS
- DN 130:119566
- TI Antioxidant efficacy of phytoestrogens in chemical and biological model systems
- AU Mitchell, Julie H.; Gardner, Peter T.; McPhail, Donald B.; Morrice, Philip C.; Collins, Andrew R.; Duthie, Garry G.
- CS Division of Micronutrients and Lipid Metabolism, Rowett Research Institute, Aberdeen, AB21 9SB, UK
- SO Archives of Biochemistry and Biophysics (1998), 360(1), 142-148 CODEN: ABBIA4; ISSN: 0003-9861
- PB Academic Press
- DT . Journal
- LA English
- RE.CNT 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 36 OF 97 CAPLUS COPYRIGHT 2002 ACS

- AN 1998:772089 CAPLUS
- DN 130:94601
- TI Characterization of phenolic **antioxidants** from mate (Ilex paraguayensis) by liquid chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry
- AU Carini, M.; Facino, R. Maffei; Aldini, G.; Calloni, M.; Colombo, L.
- CS Istituto Chimico Farmaceutico Tossicologico, Milan, 20131, Italy
- SO Rapid Communications in Mass Spectrometry (1998), 12(22), 1813-1819 CODEN: RCMSEF; ISSN: 0951-4198
- PB John Wiley & Sons Ltd.
- DT Journal
- LA English
- RE.CNT 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD ALL CITATIONS AVAILABLE IN THE RE FORMAT
- L13 ANSWER 37 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1998:127661 CAPLUS
- DN 128:215487
- TI Plant phenolics and health effects
- AU Bitsch, R.
- CS Institut Ernahrung, Umwelt Friedrich-Schiller-Universitat Jena, Lehrstuhl Humaneernahrung, Jena, 07743, Germany
- SO Vitamine und Zusatzstoffe in der Ernaehrung von Mensch und Tier, Symposium, 6th, Jena, Sept. 24-25, 1997 (1997), 113-122. Editor(s): Schubert, Rainer. Publisher: Friedrich-Schiller-Universitaet Jena, Biologisch-Pharmazeutische Fakultaet, Institut fuer Ernaehrung und Umwelt, Jena, Germany. CODEN: 65SGAF
- DT Conference; General Review
- LA German
- L13 ANSWER 38 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1996:55205 CAPLUS
- DN 124:115826
- TI Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines
- AU Teissedre, Pierre L.; Frankel, Edwain N.; Waterhouse, Andrew L.; Peleg, Hanna; German, J. Bruce
- CS Dep. Viticulture and Enol., Univ. California, Davis, CA, 95616, USA
- SO Journal of the Science of Food and Agriculture (1996), 70(1), 55-61 CODEN: JSFAAE; ISSN: 0022-5142
- PB Wilev
- DT Journal
- LA English
- L13 ANSWER 39 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1995:582977 CAPLUS
- DN 123:190934
- TI In vivo effect of dietary factors on the molecular action of aflatoxin B1: Role of non-nutrient phenolic compounds on the catalytic activity of liver fractions
- AU Aboobaker, V. S.; Balqi, A. D.; Bhattacharya, R. K.
- CS Radiation Biology and Biochemistry Division, Bhabha Atomic Research Centre, Bombay, 400 085, India
- Centre, Bombay, 400 085, India SO In Vivo (1994), 8(6), 1095-8 CODEN: IVIVE4; ISSN: 0258-851X
- DT Journal
- LA English
- L13 ANSWER 40 OF 97 CAPLUS COPYRIGHT 2002 ACS
- AN 1995:554235 CAPLUS
- DN 123:25427
- TI The red wine phenolics trans-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: Implications

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nepalense Sweet
ΑU
     Abe, Katuo
     Kochi Women's Univ., Kochi, 780, Japan
CS
     Kochi Joshi Daigaku Kiyo, Shizen Kagaku-hen (1992), 40, 25-33
SO
     CODEN: KJDSA6; ISSN: 0452-2486
DT
     Journal
     Japanese
LA
L13 ANSWER 46 OF 97 CAPLUS COPYRIGHT 2002 ACS
     1992:611408 CAPLUS
AN
     117:211408
DN
     Efficacy of various flavonoids and simple phenolics in
ΤI
     prevention of nutritional myopathy in the chick
     Jenkins, K. J.; Collins, F. W.; Hidiroglou, M.
ΑU
     Cent. Food Anim. Res., Agric. Canada, Ottawa, ON, K1A 0C6, Can.
CS
     Poultry Science (1992), 71(9), 1577-80
SO
     CODEN: POSCAL; ISSN: 0032-5791_____
DΤ
     Journal
LA
     English
L13 ANSWER 47 OF 97 CAPLUS COPYRIGHT 2002 ACS
     1992:590440 CAPLUS
DN
     117:190440
     Protection by albumin against the pro-oxidant actions of phenolic dietary
ΤI
     components
     Smith, C.; Halliwell, B.; Aruoma, O. I.
ΑU
     King's Coll., Univ. London, Strand/London, WC2R 2LS, UK
CS
     Food and Chemical Toxicology (1992), 30(6), 483-9
SO
     CODEN: FCTOD7; ISSN: 0278-6915
DT
     Journal
LA
     English
L13 ANSWER 48 OF 97 CAPLUS COPYRIGHT 2002 ACS
     1990:137701 CAPLUS
ΑN
DN
     112:137701
     Antioxidant activity of phenolic substances in aqueous and lipid systems
ΤI
     Barrera-Arellano, D.; Esteves, W.
ΑU
     Fac. Eng. Aliment., UNICAMP, Campinas, 13.083, Brazil
CS
     Ciencia e Tecnologia de Alimentos (Campinas, Brazil) (1989), 9(2), 107-14
SO
     CODEN: CTALDN; ISSN: 0101-2061
DT
     Journal
LA
     Portuguese
     ANSWER 49 OF 97 CAPLUS COPYRIGHT 2002 ACS
(L13
ΑN
     1989:567353 CAPLUS
DN
     111:167353
ΤI
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     quercetin, gossypol and myricetin. Effects on lipid peroxidation,
     hydroxyl radical generation and bleomycin-dependent damage to DNA
     Laughton, Miranda J.; Halliwell, Barry; Evans, Patricia J.; Hoult, J.
ΑU
     Robin S.
     King's Coll., Univ. London, London, WC2R 2LS, UK
CS
     Biochemical Pharmacology (1989), 38(17), 2859-65
SO
     CODEN: BCPCA6; ISSN: 0006-2952
DT
     Journal
LA
     English
    ANSWER 50 OF 97 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
L13
     2002:537654 BIOSIS
ΑN
DN
     PREV200200537654
     Antioxidant activities and phenolic composition of extracts from Greek
TΙ
     oregano, Greek sage, and summer savory.
     Exarchou, V.; Nenadis, N.; Tsimidou, M. (1); Gerothanassis, I. P.;
ΑU
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Troganis, A.; Boskou, D.

- CS (1) Laboratory of Food Chemistry and Technology, School of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, GR-54124: tsimidou@chem.auth.gr Greece
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- DT Article
- LA English
- L13 ANSWER 51 OF 97 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
- AN 2001:532336 BIOSIS
- DN PREV200100532336
- TI High-throughput fluorescence screening of antioxidative capacity in human serum.
- AU Mayer, Birgit; Schumacher, Martin; Brandstatter, Helga; Wagner, Franz S.; Hermetter, Albin (1)
- CS (1) Department of Biochemistry, Technische Universitat-Graz, A-8010, Graz: albin.hermetter@tu-graz.at Austria
- SO Analytical Biochemistry, (October 15, 2001) Vol. 297, No. 2, pp. 144-153. print.
 ISSN: 0003-2697.
- DT Article
- LA English
- SL English
- L13 ANSWER 52 OF 97 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
- AN 2001:261115 BIOSIS
- DN PREV200100261115
- TI Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography.
- AU Chen, Hao; Zuo, Yuegang (1); Deng, Yiwei
- CS (1) Department of Chemistry and Biochemistry, University of Massachusetts, Dartmouth, North Dartmouth, MA, 02747: yzuo@umassd.edu USA
- SO Journal of Chromatography A, (13 April, 2001) Vol. 913, No. 1-2, pp. 387-395. print. ISSN: 0021-9673.
- DT Article
- LA English
- SL English
- L13 ANSWER 53 OF 97 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
- AN 2001:90186 BIOSIS
- DN PREV200100090186
- TI LC coupled to ion-trap MS for the rapid screening and detection of polyphenol antioxidants from Helichrysum stoechas.
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- CS Dep. of Food Sci., Univ. of Reading, London Road, Reading RG1 5AQ, UK
- SO Journal of the Science of Food and Agriculture, (1980), 31 (7) 646-650, 8 ref.
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- Lehrstuhl fuer Lebensmittelchem. der Tech. Univ., 3, Hanover, Wunstorfer CS Strasse 14, Federal Republic of Germany Fette, Seifen, Anstrichmittel, (1973), 75 (8) 499-504, 62 ref.
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LA German

SL English; French; Russian

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 AN
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PΒ
     Institute of Food Technologists
DT
     Journal
     English
LA
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ΤI
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PΒ
     American Chemical Society
DT
     Journal
     English
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AN
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     McPhail, Donald B.; Gardner, Peter T.; Duthie, Garry G.; Steele, Gordon
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     Journal
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